NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 430

TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. DIRECT BLUE 218

(CAS NO. 28407-37-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from the NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

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(FEED STUDIES)

A desalted commercial dye containing approximately 60% copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, 1% sodium chloride, 9% water, and 30% unknown

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P.O. Box 12233
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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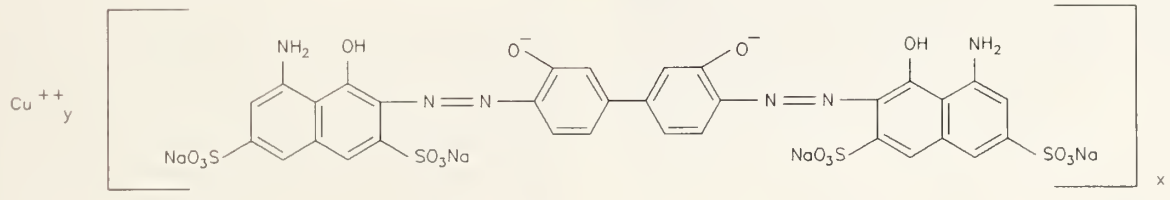
CONTENTS

ABSTRACT

C.I. DIRECT BLUE 218

CAS No. 28407-37-6



Major Component of C.I. Direct Blue 218

A copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis [5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt

Chemical Formula: $(C_{32}H_{18}N_6O_{16}S_4Na_4)_xCu_y$ Molecular Weight: Approx. 1,090
(assumes 2 copper ions per molecule)

Synonyms: cuprate(4-), [μ-[[3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato]](8-)]]di-, tetrasodium; copper, [tetrahydrogen-3,3′-[(3,3′-dihydroxy-4,4′-biphenylylene) bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato](4-)]di-, tetrasodium salt; 1-naphthol-3,6-disulfonic acid, 2,2′-(3,3′-dihydroxy-4,4′-biphenylylenebisazo)bis[8-amino-, dicopper deriv., tetrasodium salt

Trade Names: Amanil Supra Blue 9GL; Carta Blue VP; Fastusol Blue 9GLP; Pontamine Bond Blue B; Solantine Blue 10GL; Pontamine Fast Blue 7GLN;

C.I. Direct Blue 218 is a copper chelated dye used for cellulose, acetate, nylon, silk, wool, tissue, papers, and textile goods with a urea-formaldehyde finish. C.I. Direct Blue 218 is one of five chemicals/dyes that are part of the National Toxicology Program's Benzidine Dye Initiative, established to determine the toxicity and carcinogenicity of representative benzidine congeners, congener-derived dyes, and benzidine-derived dyes. Industrial grade C.I. Direct Blue 218 was selected for study because of its widespread use. Because of the high salt content, the dye was desalted prior to use. Toxicology and carcinogenesis studies were conducted by administering C.I. Direct Blue 218 in feed to groups of male and female F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and *Drosophila melanogaster*.

14-Day Study in Rats

Groups of five male and five female F344/N rats were fed diets containing 0, 1,000, 3,000, 7,000, 15,000, or 30,000 ppm C.I. Direct Blue 218. All rats survived until the end of the study. Rats receiving 30,000 ppm lost weight, and the mean body weight gain of males receiving 15,000 ppm was significantly lower than that of the controls. Feed consumption by rats receiving 30,000 ppm was lower than that by the controls. Decreased organ weights at the 30,000 ppm level were related to the decreased body weights at this exposure level.

14-Day Study in Mice

Groups of five male and five female mice were fed diets containing 0, 1,000, 3,000, 7,000, 15,000, or

30,000 ppm C.I. Direct Blue 218. All mice survived until the end of the study. The final mean body weight of males receiving 30,000 ppm was 25% lower than that of controls and that of 30,000 ppm females was 20% lower than that of controls. Feed consumption by exposed and control groups was similar except for the 15,000 and 30,000 ppm groups. Feed spillage, due to reduced palatability, precluded the accurate determination of feed consumption by these two groups. Male and female mice receiving 30,000 ppm appeared hyperactive and emaciated during the last week of the study. Decreased organ weights were noted at 30,000 ppm and were attributed to the decreased mean body weights at this exposure level.

13-Week Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218. All male and female rats survived until the end of the study. Rats exposed to 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218 received approximate daily doses of 200, 600 or 1,300 mg dye/kg body weight (males) and 200, 800, or 1,400 mg/kg (females). The final mean body weight of male rats receiving 20,000 ppm was 24% lower than that of the controls and the final mean body weight of female rats receiving 20,000 ppm was 15% lower than that of the controls. Feed consumption by exposed and control groups was similar except in the 20,000 ppm groups where feed spillage was noted. Absolute and relative kidney weights of rats receiving 10,000 or 20,000 ppm were significantly greater than those of controls. Significantly decreased organ weights were noted, particularly in the 20,000 ppm groups, and were attributed to the lower mean body weights at this exposure level.

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values in male and female rats receiving 10,000 and 20,000 ppm were significantly lower than those of controls. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female rats receiving 20,000 ppm were significantly higher than those of controls, which is consistent with hepatocellular injury. Male rats receiving 10,000 ppm and male and female rats receiving 20,000 ppm had hepatic lesions consisting of intracytoplasmic pigment in periportal Kupffer cells, minimal to mild individual hepatocyte necrosis, increased numbers of binucleated and multinucleated hepatocytes, and minimal bile duct hyperplasia. Male and female rats receiving 20,000 ppm had yellow-green pigment within the cytoplasm of proximal convoluted tubules of the kidney. Microconcretions of mineral were observed along the corticomedullary junction of the kidney in most female rats, but the numbers of microconcretions in kidney sections were increased in females that received 20,000 ppm.

13-Week Study in Mice

Groups of 10 male and 10 female $B6C3F_1$ mice were fed diets containing 0, 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218. There were no deaths attributed to C.I. Direct Blue 218. Mice exposed to 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218 received approximate daily doses of 400, 1,500, or 3,600 mg dye/kg body weight (males) and 400, 1,800, or 4,000 mg/kg (females). The final mean body weight of males that received 20,000 ppm was 24% lower than that of the controls, and the final mean body weight of females that received 20,000 ppm was 14% lower than that of controls. Feed consumption by exposed mice was similar to that by controls except in the 20,000 ppm groups where feed spillage was noted. Significant differences in organ weights were noted at 20,000 ppm which were attributed primarily to the lower mean body weights in these exposure groups.

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values were significantly lower in males and females receiving 10,000 and 20,000 ppm. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female mice receiving 10,000 and 20,000 ppm were significantly higher than those of controls, indicating hepatic injury. Male and female mice receiving 20,000 ppm had hepatic lesions consisting of centrilobular hepatocyte hypertrophy and karyomegaly, multifocal individual hepatocyte necrosis, oval cell proliferation, and periportal Kupffer cells with intracytoplasmic pigment. Males and females receiving 20,000 ppm also had increased numbers of pigmented macrophages within the red pulp of the spleen.

2-Year Study in Rats

The doses selected for the 2-year study of C.I. Direct Blue 218 were based on the lower final mean body weights and the occurrence of hepatic lesions in the

20,000 ppm groups in the 13-week study. Groups of 60 male and 60 female rats were fed diets containing 0, 1,000, 3,000, or 10,000 ppm C.I. Direct Blue 218 for 103 weeks. Nine or 10 rats from each group were evaluated after 15 months.

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

Survival of female rats receiving 10,000 ppm was slightly, but not significantly, lower than that of the controls. Mean body weights of male and female rats in the 10,000 ppm groups were approximately 5% to 14% lower than those of the controls after week 15, and the final mean body weights of male and female rats at this level were 11% and 9% lower than those of the controls, respectively. Feed consumption by exposed male and female rats was similar to that by the controls and was estimated to deliver daily doses of 40, 120, and 440 mg dye/kg body weight to males and 50, 140, and 470 mg/kg to females. No chemical-related clinical signs of toxicity were noted.

Hematology and Clinical Chemistry

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values in 10,000 ppm female rats were significantly lower than those of controls, while in males only the mean erythrocyte hemoglobin value was significantly lower. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female rats receiving 10,000 ppm were significantly higher than those of the controls at the 15-month interim evaluation.

Pathology Findings

Squamous cell papillomas of the oral mucosa (pharynx) occurred in five males receiving 10,000 ppm but not in the lower exposure groups or in controls. A squamous cell carcinoma occurred in one 10,000 ppm male and a benign basosquamous tumor was observed in another. The incidence of oral mucosal neoplasms in the 10,000 ppm males was significantly greater than that in controls and exceeded the range observed in untreated historical controls (10/1,253, 0.8%; range 0%-4%). These neoplasms were considered chemical related.

Administration of C.I. Direct Blue 218 to rats produced significantly increased incidences of forestomach basal cell hyperplasia in males receiving 3,000 or 10,000 ppm (0 ppm, 0/50; 1,000 ppm, 2/50; 3,000 ppm, 10/50; 10,000 ppm, 19/50) and in females receiving 10,000 ppm (1/50, 1/49, 5/50, 11/49). Further, there were marginal increased incidences of focal squamous hyperplasia in the 3,000 and 10,000 ppm males (1/50, 1/50, 6/50, 4/50). Squamous cell papillomas of the forestomach were seen in two 3,000 ppm males and in one 10,000 ppm male; no papillomas were observed in the controls. A squamous cell carcinoma was also seen in one 3,000 ppm male. Because of the uncommon occurrence of forestomach neoplasms in untreated control male rats (4/1,253, 0.3%; range 0%-2%) and the slight increase in the incidence of focal hyperplasia, these neoplasms may have been chemical related.

The incidence of uterine endometrial stromal polyps in each exposed group of female rats was significantly greater than that of the controls (1/50, 12/50, 10/50, 10/50). Because the incidences in the exposed groups did not increase in a dose-related manner and the incidence in the controls was unusually low (historical incidence: 205/1,251, 16.4%; range 2%-30%), the higher incidence of stromal polyps in the exposed groups was not considered chemical related.

2-Year Study in Mice

The dose selection for the 2-year study was based on the lower final mean body weights and the liver lesions observed at the 20,000 ppm level in the 13-week study. Groups of 60 male and 60 female mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm C.I. Direct Blue 218 for 103 weeks. Nine or 10 mice from each exposure group were evaluated after 15 months.

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

Survival of exposed male and female mice was similar to that of the controls. Mean body weights of male and female mice receiving 10,000 ppm were 10% to 29% lower than those of the controls during most of the study, and the final mean body weights in these groups were 19% lower than that of the controls for males and 27% lower than that of the controls for females. Feed consumption by exposed mice was similar to that by controls and the diets were estimated to deliver daily doses of approximately 120, 360, and 1,520 mg of dye/kg body weight to males and 140, 470, and 2,050 mg/kg to females. No chemical-related clinical signs of toxicity were noted.

Hematology and Clinical Chemistry

Hematocrit, hemoglobin, and mean erythrocyte volume values in males and females receiving 10,000 ppm were significantly lower than those of the controls. Serum levels of alanine aminotransferase and/or sorbitol dehydrogenase values in male and female mice that received 10,000 ppm were significantly higher than those of controls, which is consistent with hepatocellular damage.

Pathology Findings

The administration of C.I. Direct Blue 218 to mice produced significantly increased incidences of hepatocellular adenoma (0 ppm, 16/50; 1,000 ppm, 19/50; 3,000 ppm, 17/50; 10,000 ppm, 40/50) and hepatocellular carcinoma (7/50, 3/50, 8/50, 17/50) in males receiving 10,000 ppm, and a significantly increased incidence of hepatocellular adenoma in females receiving 3,000 or 10,000 ppm (7/49, 12/50, 17/49, 41/49). In females that received 10,000 ppm, the incidence of hepatocellular carcinoma was marginally increased. Consistent with these findings, the incidence of hepatocellular foci of cytologic alteration, a preneoplastic lesion, was also increased in males and females in the 10,000 ppm groups. The increased incidences of hepatocellular foci, adenomas, and carcinomas were considered chemical related.

Uncommon renal tubule neoplasms also occurred at low incidences in male mice receiving C.I. Direct Blue 218, but not in controls. Renal tubule adenomas were seen in two males receiving 1,000 ppm, one male receiving 3,000 ppm, and one male receiving 10,000 ppm. A renal tubule carcinoma was also seen in one male that received 1,000 ppm. Because renal tubule neoplasms are uncommon in male mice (4/1,366, 0.3%; range 0%-2%), these neoplasms may have been chemical related.

Carcinomas of the small intestine occurred in four male mice receiving 10,000 ppm. One was observed at the 15-month interim evaluation, while the other three were observed in mice at the end of the study. One control male mouse also had a carcinoma of the small intestine. Because of the uncommon occurrence of small intestine neoplasms in untreated male mice (12/1,374, 0.9%; range 0%-4%), the slightly higher incidence of these neoplasms in males receiving 10,000 ppm may have been chemical related. Carcinomas of the small intestine also occurred in one 3,000 ppm and one 10,000 ppm female, but the low incidences precluded drawing an association with chemical administration.

Genetic Toxicology

C.I. Direct Blue 218 was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 tested with and without exogenous metabolic activation (S9). It was also tested in a modified *Salmonella* test protocol which employed reductive metabolism supplied by flavin mononucleotide or rat cecal bacteria, followed by oxidative metabolism; results of this test using strain TA1538 were also negative. C.I. Direct Blue 218 induced a small but significant increase in sister chromatid exchanges in Chinese hamster ovary cells at the highest dose tested without S9. No increase in chromosomal aberrations were observed in Chinese hamster ovary cells with or without S9. C.I. Direct Blue 218 did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*.

Conclusions

Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity** of C.I. Direct Blue 218 in male F344/N rats based on the occurrence of pharyngeal neoplasms. Squamous cell neoplasms of the forestomach may have been chemical related. There was *no evidence of carcinogenic activity* of C.I. Direct Blue 218 in female F344/N rats given 1,000, 3,000, or 10,000 ppm. There was *clear evidence of carcinogenic activity* of C.I. Direct Blue 218 in male and female B6C3F$_1$ mice based on increased incidences of hepatocellular adenomas and carcinomas. The occurrence of a few neoplasms of the kidney and small intestine in male mice may have been related to C.I. Direct Blue 218 treatment.

The administration of C.I. Direct Blue 218 produced an increased incidence of forestomach basal cell hyperplasia in rats and hepatocellular foci of cytologic alteration in mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of C.I. Direct Blue 218

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 1,000, 3,000, or 10,000 ppm in feed (0, 40, 120, or 440 mg/kg)	0, 1,000, 3,000, or 10,000 ppm in feed (0, 50, 140, or 470 mg/kg)	0, 1,000, 3,000, or 10,000 ppm in feed (0, 120, 360, or 1,520 mg/kg)	0, 1,000, 3,000, or 10,000 ppm in feed (0, 140, 470, or 2,050 mg/kg)
Body weights	High-dose group lower than controls	High-dose group lower than controls	High-dose group lower than controls	High-dose group lower than controls
2-Year survival rates	30/50, 25/50, 29/50, 24/51	35/51, 29/51, 31/50, 25/50	44/50, 46/50, 42/50, 45/50	37/49, 40/50, 46/49, 38/49
Nonneoplastic effects	Forestomach: basal cell hyperplasia (0/50, 2/50, 10/50, 19/50)	Forestomach: basal cell hyperplasia (1/50, 1/49, 5/50, 11/49)	Liver: eosinophilic foci (13/50, 12/50, 10/50, 28/50); all foci (combined) 22/50, 22/50, 16/50, 32/50	Liver: eosinophilic foci (11/49, 7/50, 11/49, 21/49); all foci (combined) 13/49, 11/50, 17/49, 29/49
Neoplastic effects	Pharynx: squamous cell papilloma (0/50, 0/50, 0/50, 5/50); squamous cell carcinoma (0/50, 0/50, 0/50, 1/50); basosquamous tumor benign (0/50, 0/50, 0/50, 1/50)	None	Liver: hepatocellular adenoma (16/50, 19/50, 17/50, 40/50); hepatocellular carcinoma (7/50, 3/50, 8/50, 17/50)	Liver: hepatocellular adenoma (7/49, 12/50, 17/49, 41/49); hepatocellular carcinoma (5/49, 5/50, 6/49, 12/49)
Uncertain findings	Forestomach: squamous cell papilloma (0/50, 0/50, 2/50, 1/50); squamous cell carcinoma (0/50, 0/50, 1/50, 0/50)	None	Kidney (renal tubule): adenoma (0/50, 2/50, 1/50, 1/50); carcinoma (0/50, 1/50, 0/50, 0/50); adenoma or carcinoma (0/50, 3/50, 1/50, 1/50) Small intestine: carcinoma (1/50, 0/50, 0/50, 3/50)	None
Level of evidence of carcinogenic activity	Some evidence	No evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537			
Salmonella typhimurium with reductive metabolism:	Negative in strain TA1538			
Sister chromatid exchanges Chinese hamster ovary cells *in vitro*:	Weakly positive without S9; negative with S9			
Chromosomal aberrations Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Sex-linked recessive lethal mutations *Drosophila melanogaster*:	Negative when administered in feed or by injection			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on C.I. Direct Blue 218 on December 1, 1992, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 1, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Direct Blue 218 received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of C.I. Direct Blue 218 by discussing the use and rationale for study (as part of the NTP Benzidine Dye Initiative), describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions were *some evidence of carcinogenic activity* for male F344/N rats, *no evidence of carcinogenic activity* for female F344/N rats, and *clear evidence of carcinogenic activity* for male and female B6C3F_1 mice.

Dr. Davidson, a principal reviewer, agreed with the proposed conclusions. She said the background information on the metabolism, toxicity, and carcinogenicity of benzidine and benzidine-congener based dyes was detailed and appeared representative of the available literature.

Mr. Beliczky, the second principal reviewer, agreed with the proposed conclusions. He thought a tabular reference chart comparing results for various benzidine dye derivatives should be included (Table 21).

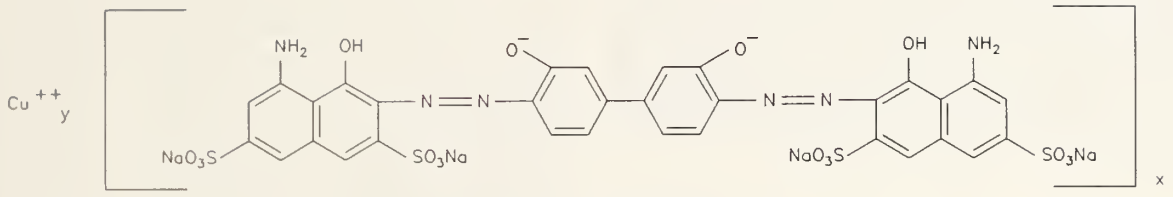
Dr. Ward commented on the incidence of three kidney neoplasms in male mice at the lowest dose, wondering why this finding did not fall under *clear evidence* as these are rare neoplasms and there was a corresponding lack of renal toxicity. Dr. Dunnick responded that the lack of a dose response and the lack of statistical significance compared with the controls led to the conclusion that these were only uncertain findings. Dr. Davis questioned the rationale for the oral route of administration being selected to mimic exposure in the home and workplace, suggesting that dermal exposure was more likely. Dr. Ryan noted the teratogenic effects reported for the benzidine-based dyes and suggested reproductive and developmental toxicology studies would be appropriate. Dr. Dunnick reported that at the time the benzidine-based dye studies were initiated, potential for carcinogenesis was a primary concern, but agreed that reproductive effects should be, and are receiving, increasing priority.

Dr. Davidson moved that the Technical Report on C.I. Direct Blue 218 be accepted with the revisions discussed and with the conclusions as written for male rats, *some evidence of carcinogenic activity,* for female rats, *no evidence of carcinogenic activity*, and for male and female mice, *clear evidence of carcinogenic activity.* Mr. Beliczky seconded the motion, which was accepted unanimously with ten votes.

INTRODUCTION

C.I. DIRECT BLUE 218

CAS No. 28407-37-6



Major Component of C.I. Direct Blue 218

A copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis [5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt

Chemical Formula: $(C_{32}H_{18}N_6O_{16}S_4Na_4)_xCu_y$ Molecular Weight: Approx. 1,090 (assumes 2 copper ions per molecule)

Synonyms: cuprate(4-), [μ-[[3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato]](8-)]di-, tetrasodium; copper, [tetrahydrogen-3,3′-[(3,3′-dihydroxy-4,4′-biphenylylene) bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato](4-)]di-, tetrasodium salt; 1-naphthol-3,6-disulfonic acid, 2,2′-(3,3′-dihydroxy-4,4′-biphenylylenebisazo)bis[8-amino-, dicopper deriv., tetrasodium salt

Trade Names: Amanil Supra Blue 9GL; Carta Blue VP; Fastusol Blue 9GLP; Pontamine Bond Blue B; Solantine Blue 10GL; Pontamine Fast Blue 7GLN;

Chemical and Physical Properties

C.I. Direct Blue 218 is a dark blue solid with a salt content of approximately 15%. C.I. Direct Blue 218 is produced by coupling one mole of *o*-dianisidine (3,3′-dimethoxybenzidine) to two moles of 4-amino-5-hydroxy-2,7-naphthalene disulfonic acid under alkaline pH conditions followed by metallizing and elimination of methyl groups from the methoxides to form the copper complex (*Kirk-Othmer*, 1978). The lot of dye selected for study was characteristic of the product currently used by the dye industry and the chemical to which workers were potentially exposed.

The dye was desalted by dialysis to reduce the salt content to approximately 1% to 3%, after which the dye was a dark blue amorphous powder. The major component accounted for approximately 60% of the dye and the copper content of the dye was approximately 9%. More than 9 minor components (>1%) were present in the dye and although it was not possible to determine the definite structure of these impurities, the reducible azo bond appeared to be present in the majority of the impurities. Because of limited solubility in water, the dye was administered to animals in feed.

Use and Human Exposure

C.I. Direct Blue 218 is used as a dye for cellulose, acetate, nylon, silk, wool, tissue, papers, and textile goods with a urea-formaldehyde finish. Boeninger (1980) reported the total use of benzidine-based dyes as follows: 40% is used to color paper, 25% to color textiles, 15% in leather, and 20% in diverse applications in the petroleum, rubber, plastics, wood, soap, fur, and hair dye industries. The U.S. production of C.I. Direct Blue 218 is 3.3×10^8 grams per year

(USITC, 1985). Information on the amount of dye imported was not reported in the open literature.

From a survey conducted from 1981 to 1983, the National Institute for Occupational Safety and Health (NIOSH) estimated that a total of 12,290 workers might come into contact with C.I. Direct Blue 218 in the textile and paper industries (NIOSH, 1990). Industrial exposure to dyes may occur through inhalation of dust or mist, accidental ingestion, or direct contact with the skin. The potential for exposure to C.I. Direct Blue 218 occurs during the manufacturing process, from products containing the dye, or from contaminated water supplies (USEPA, 1980; Fishbein, 1981; NIOSH, 1983).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Metabolism studies of the azo dyes have generally involved oral administration of the dye to rodents or dogs followed by urinalysis to determine the presence of the dye or metabolite. The dyes studied included representative dyes derived from the various parent congeners including those made from benzidine, 3,3′-dichlorobenzidine, 3,3′-dimethoxybenzidine, 2,2′-disulfobenzidine, or 3,3′-dimethylbenzidine. While there have been no metabolism studies of C.I. Direct Blue 218 reported in the literature, information on the metabolism of structurally related benzidine dyes shows that the azo bond in these dyes is reduced by bacterial enzymes in the intestine, and a small amount of orally administered dyes is absorbed and subsequently metabolized in the liver (Lynn *et al.*, 1984).

Metabolism of 3,3′-dimethylbenzidine-based and 3,3′-dimethoxybenzidine-based dyes to 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine occurs in dogs and rats (Lynn *et al.*, 1980). The urine of dogs and rats was examined for the presence of benzidine metabolites after a single oral dose (100 mg/kg) of a series of azo dyes. The dyes administered included benzidine-derived dyes (Direct Orange 1 or 8, Direct Green 1, Direct Brown 2, Direct Black 4, Direct Blue 2, or Direct Red 28); dimethoxybenzidine-derived dyes (Direct Blue 15 or Blue 1); and dimethylbenzidine-derived dyes (C.I. Direct Blue 25, C.I. Acid Red 114, Direct Red 2 or 39). The parent congeners for the dyes studied (benzidine, 3,3′-dimethoxybenzidine, or 3,3′-dimethylbenzidine) were detected in the urine of dogs and rats. In addition, N-acetylated metabolites were also detected in substantial quantities in rat urine while N-acetylated metabolites were not detected in dog urine, which is consistent with the observation that dogs do not acetylate aromatic amines (Lynn *et al.*, 1980).

Studies by other investigators using other representative benzidine or benzidine-congener dyes have also shown that the parent congener is excreted in the urine (Nony and Bowman, 1980; Nony *et al.*, 1980; Bowman *et al.*, 1982; Bos *et al.*, 1984, 1986). Bowman *et al.* (1982) administered 12 mg ^{14}C-labeled C.I. Direct Blue 15 per kg body weight or molar equivalent doses of 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine to male F344 rats by oral gavage. Approximately 19% of the label was recovered in the urine of rats given C.I. Direct Blue 15; 35% to 40% of the label was recovered in the urine of rats administered 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine.

The reductive cleavage of benzidine-congener azo dyes is thought to occur primarily by bacterial azoreductases in the intestinal tract (Martin and Kennelly, 1981; Cerniglia *et al.*, 1982; Brown and Dietrich, 1983; Bos *et al.*, 1984, 1986). Mammalian tissues such as the liver also contain azoreductases, but because of the highly polar nature of the benzidine dyes, mammals do not absorb the intact dyes to any great extent, and reduction of the azo bond takes place primarily in the intestine (Walker, 1970). Studies in rats using representative azo dyes showed that when the dye was administered by intrasplenic infusion, most of the dye was excreted unchanged in the bile, indicating that liver azo reductases do not appear to play a major role in cleavage of the azo linkages in these dyes (Radomski and Mellinger, 1962).

Benzidine, benzidine congeners, and benzidine-based dyes are believed to be carcinogenic only following metabolic activation. Evidence for the increased mutagenicity of benzidine following metabolic activation was provided by Lynn *et al.* (1984) and others who reported that the glucuronide of N-hydroxy-N,N-diacetylbenzidine was as much as 100 times more mutagenic in the *Salmonella*/liver S9 assay than benzidine. Lynn *et al.* (1984) have shown that after oral administration of benzidine to rats, the major metabolites in the urine were glucuronide conjugates

of benzidine, N-acetylbenzidine, N,N′-diacetylbenzidine, 3-hydroxy-N,N′-diacetylbenzidine, and free N-acetyl- and N,N′-diacetylbenzidine. Biliary excretion of N-acetylated benzidine metabolites occurred in rats, while N-acetylated products of benzidine were not detected in the bile or urine from dogs. The major bile or urine metabolites found in the dog were conjugated products of 3-hydroxybenzidine (Lakshmi *et al.*, 1990).

The presence of DNA alkylating products in tissues of rodents are generally believed to be involved with the occurrence of cancer. In the rodent liver benzidine is metabolized to N-acetylbenzidine and oxidized to N′-hydroxy-N-acetylbenzidine. Formation of the latter compound is thought to initiate hepatic neoplasms in rodents. Martin *et al.* (1982) administered benzidine to rats and identified N-(deoxyguanosin-8-yl)-N′-acetylbenzidine as a product of DNA alkylation. It is possible that components in the C.I. Direct Blue 218 dye may also form a similar DNA alkylation product, although studies to determine the extent to which such metabolites are formed have not been conducted.

Humans

There is no information on the metabolism of C.I. Direct Blue 218 in humans, but studies of the related chemical benzidine have shown that benzidine exposure is related to the occurrence of urinary bladder cancer (IARC, 1972a,b). Benzidine metabolites have been found in the urine of workers exposed to benzidine dyes in industrial settings (Genin, 1977; Boeninger, 1980; NIOSH, 1980).

Studies to elucidate the benzidine metabolite responsible for causing cancer in the urinary bladder of humans have used the dog as a model system because the N-acetylation of benzidine is not a predominant route of metabolism in either the dog or human (Lower and Bryan, 1973). Benzidine N-glucuronide had recently been identified as a new metabolite of benzidine in the dog and was found in the urine, bile, and plasma (Babu *et al.*, 1992). It is hypothesized that in humans and dogs the liver detoxifies benzidine by forming benzidine N-glucuronide which is excreted into plasma, filtered by the kidney, and accumulated in urine. Acidic urine hydrolyzes N-glucuronide to benzidine. Urinary bladder prostaglandin H synthetase activates benzidine (Flammang *et al.*, 1989).

Several other studies have shown that metabolism of benzidine is necessary for the formation of the ultimate carcinogen in the urinary bladder. Wang *et al.* (1990), using a rat implanted with a heterotropic bladder that had been injected with a series of benzidine dyes, found that induction of bladder cancer occurred with N-hydroxy metabolites of benzidine, but not with benzidine alone.

Lower benzidine UDP-glucuronosyltransferase activity in the rodent may contribute to the lack of formation of benzidine glucuronide, transport of benzidine to the urinary bladder, and formation of neoplasms at this site (Babu *et al.*, 1992).

TOXICITY

Experimental Animals

There are no toxicity studies reported in the literature for C.I. Direct Blue 218. Unpublished reports submitted to the USEPA under the Toxic Substances Control Act (TSCA) Program by Scientific Associates, Inc. (St. Louis, MO), reported that Solantine Blue 10GL (C.I. Direct Blue 218) had acute oral LD_{50} values of 3.3 g/kg in SASCO rats and 4 g/kg in New Zealand albino rabbits. Acute dermal toxicity was not observed when doses of more than 8 g/kg were applied to the backs of New Zealand albino rabbits. Details on the chemical composition of the dye were not reported.

Reproductive toxicity studies have not been reported for C.I. Direct Blue 218, but Wilson (1955) studied the teratogenic potential of several 3,3′-dimethoxybenzidine (DMOB) or 3,3′-dimethylbenzidine (DMB) based dyes in albino rats by injecting pregnant females with a 1% aqueous solution of each dye on days 7, 8, and 9 of gestation. Trypan Blue (DMB) was the most potent teratogen, causing malformations in 49% of living offspring, followed by Evans Blue (DMB) which caused abnormalities in 14% of offspring, Niagara Blue 4B (DMOB), which caused abnormalities in 4% of offspring, and Niagara Sky Blue 6B (DMOB) which caused abnormalities in 3% of offspring. The teratogenic effects of the azo dyes were confirmed in a series of studies by Beaudoin and Pickering (1960), Lloyd *et al.* (1965), Beck and Lloyd (1966), Lloyd and Beck (1966), and Beaudoin (1968). Although the purity and chemical characterization of the dyes used were not reported, the abnormalities

were generally similar to common spontaneous malformations such as anencephaly, hydrocephaly, and spina bifida.

Humans

No information on the toxicity of C.I. Direct Blue 218 in humans has been reported.

CARCINOGENICITY

Experimental Animals

Toxicity and carcinogenicity studies of C.I. Direct Blue 218 have not been reported, but studies of its congener, 3,3′-dihydroxybenzidine have been conducted. Bonser *et al.* (1956) reported that for mice injected subcutaneously with 6 mg/kg benzidine or 3,3′-dihydroxybenzidine twice a week for 52 weeks and untreated thereafter, 7 of 60 mice receiving benzidine developed liver neoplasms while no neoplasms were observed in mice receiving 3,3′-dihydroxybenzidine. Earlier studies by Baker (1950) reported that liver neoplasms occurred in mice receiving 300 mg 3,3′-dihydroxybenzidine three times a week for 45 weeks; however the chemical used was not pure. Pliss (1963) reported that 3,3′-dihydroxybenzidine produced treatment-related neoplasms in skin and liver of rats, although this study contained no information on the purity of the chemical or details on the doses used.

Studies on structurally related chemicals including benzidine, benzidine congeners, and dyes have been reported to cause neoplasms in rodents (IARC, 1972a,b; Robens *et al.*, 1980). However, many of the early studies summarized below did not report on the purity of the chemicals, toxicologic endpoints such as survival and body weight, or histopathologic findings of all major organ systems, and, in general, used a small number of animals.

Benzidine has been identified as a carcinogen causing hepatocellular, harderian gland, and lymphoreticular neoplasms in mice (Bonser *et al.*, 1956; Vesselinovitch *et al.*, 1975; Frith and Dooley, 1976); Zymbal's gland, hepatocellular, and mammary gland carcinomas in rats (Spitz *et al.*, 1950; Griswold *et al.*, 1968); hepatocellular carcinomas, adenomas, and cholangiomas in hamsters (Saffiotti *et al.*, 1967); and urinary bladder neoplasms in dogs (Spitz *et al.*, 1950; Stula *et al.*, 1978).

3,3′-Dimethoxybenzidine treatment was associated with Zymbal's gland, small intestine, and mammary gland neoplasms in rats (Spitz *et al.*, 1950; Pliss, 1963, 1965; Pliss and Zabezhinsky, 1970). In a lifetime study of Syrian hamsters fed diets containing 1,000 ppm 3,3′-dimethoxybenzidine, the only neoplastic finding after 114 weeks of exposure was a transitional cell carcinoma of the urinary bladder (Saffioti *et al.*, 1967). Sellakumar *et al.* (1969) conducted a similar study in which 10,000 ppm 3,3′-dimethoxybenzidine was administered in feed to hamsters. Forestomach papillomas were detected in 37% of the exposed animals and in 2% of the controls, but no urinary bladder lesions were detected. Hadidian *et al.* (1968) administered 0.1 to 30 mg 3,3′-dimethoxybenzidine per day by gavage to rats for 50 weeks. A variety of neoplasms were reported, and pooled results for all exposed male and female groups included a few treatment-related neoplasms in the urinary bladder, mammary gland, skin, and Zymbal's gland.

Toxicity and carcinogenicity studies of 3,3′-dimethylbenzidine hydrochloride and 3,3′-dimethoxybenzidine dihydrochloride were conducted by Schieferstein *et al.* (1989, 1990). The BALB/c mouse was selected for study because it had previously been shown to be susceptible to chemically induced cancer of the urinary bladder (Meigs *et al.*, 1986), and the urinary bladder was a target organ for benzidine compounds in humans. 3,3′-Dimethoxybenzidine at doses of 20 to 630 ppm or 3,3′-dimethylbenzidine at doses of 5 to 140 ppm were administered to mice in drinking water for 112 to 116 weeks. No treatment-related neoplasms were reported with the exception of some lung neoplasms in male mice treated with 3,3′-dimethylbenzidine. This is in contrast to the findings reported by others in which benzidine was reported to cause liver, lung, and harderian gland neoplasms in B6C3F$_1$ mice (Vesselinovitch *et al.*, 1975; Littlefield *et al.*, 1983).

It is hypothesized that the formation of DNA adducts by metabolites of benzidine is responsible for mutations which eventually lead to cancer at various target sites (Talaska *et al.*, 1987) and may be responsible for activating oncogenes at the different target sites (Reynolds *et al.*, 1990). In the rodent liver benzidine and related chemicals and dyes are found to be acetylated (Martin *et al.*, 1982; Kennelly *et al.*, 1984). Kennelly *et al.* (1984) have identified

N-(deoxyguanosin-8-yl)-N′-acetylbenzidine as the major DNA adduct in the rat liver after benzidine treatment. In *in vitro* studies using benzidine binding to calf thymus DNA, Yamazoe *et al.* (1988) have shown that the major DNA adduct formed after benzidine treatment is N3-(deoxyguanosin-N7,C8-yl)-benzidine. The authors propose that this may be the DNA adduct responsible for mutations in the urinary bladder of humans and dogs. Additional studies are needed to quantify the degree of cross-linking caused by these adducts and the relationship to carcinogenicity at specific target sites.

In the NTP studies of 3,3′-dimethoxybenzidine dihydrochloride, 3,3′-dimethylbenzidine dihydrochloride, C.I. Direct Blue 15, and C.I. Acid Red 114 a similar spectrum of lesions in the Zymbal's gland, skin, liver, oral cavity, clitoral gland, and to a lesser extent in the intestine of F344/N rats was observed (NTP, 1990; 1991; 1992a,b). Some but not all of the four chemicals/dyes tested caused increased incidences of mesothelioma, mononuclear cell leukemia, and neoplasms of the brain, mammary gland, lung, and/or adrenal gland. Clear evidence of carcinogenic activity due to preputial gland neoplasms was reported for 3,3′-dimethoxybenzidine dihydrochloride, 3,3′-dimethylbenzidine dihydrochloride, and C.I. Direct Blue 15, but not for C.I. Acid Red 114. In all four studies, the incidences of skin and liver neoplasms were higher in male rats than in female rats. C.I. Acid Red 114 and its parent congener, 3,3′-dimethylbenzidine dihydrochloride, caused a higher incidence of liver neoplasms than did C.I. Direct Blue 15 and its parent congener, 3,3′-dimethoxybenzidine dihydrochloride.

Two of the most common sites for neoplasms were the Zymbal's gland and the epidermis of the skin. Most of the other chemicals tested by the NTP that induced either Zymbal's gland or skin neoplasms also caused neoplasms at other sites, and like these benzidine congeners/dyes, were mutagenic in the *Salmonella* assay. These carcinogenic chemicals have an aromatic amine functional group in common which is considered to be a "structural alert" for genotoxic activity (Ashby and Tennant, 1988). The benzidine congener dyes do not appear to be absorbed intact from the gastrointestinal tract, and the neoplasms resulting from treatment with either dyes or congeners may be caused by similar metabolites.

Dose response relationships for the carcinogenicity of the benzidine congener dyes and the parent amines could not be established because of the high incidence of neoplasms in most of the dosed groups which resulted in the termination of the 3,3′-dimethylbenzidine dihydrochloride, 3,3′-dimethoxybenzidine dihydrochloride, and C.I. Direct Blue 15 studies after 15 to 22 months. The C.I. Acid Red 114 studies continued for 2 years as planned.

There is evidence indicating that the activation of proto-oncogenes and the loss of specific regulatory substances, such as suppressor genes, may be distinct steps in the process of carcinogenesis (Barrett *et al.*, 1987). Activated oncogenes have been detected in only 3% of the spontaneous neoplasms in Fischer rats while activated H-*ras* or N-*ras* have been detected in 68% of epithelial neoplasms induced by benzidine congeners and derived dyes (Reynolds *et al.*, 1990). Furthermore, the presence of these activated oncogenes in several benign neoplasms suggests that *ras* activation may be an early event in the induction of neoplasms by these compounds. Thus, the activation of *ras* genes by point mutation may be a step in the induction of neoplasms, at least in rats, by this class of benzidine-derived compounds.

Humans

An increased risk of urinary bladder cancer and occupational exposure to aniline dye was first reported by Rehn in 1895. Subsequently, exposure to specific aromatic amines including 2-naphthylamine, aminobiphenyl, *o*-toluidine, and benzidine has been associated with cancer in humans (Scott, 1952; Case *et al.*, 1954; Case, 1965; IARC, 1972a,b; Yoshida and Miyakawa, 1973; Zavon *et al.*, 1973; Ward *et al.*, 1991).

There are no studies in the literature that have examined the potential risk of cancer and exposure to C.I. Direct Blue 218, although epidemiologic studies have shown an increased risk of urinary bladder neoplasms and exposure to benzidine-based dyes in general. Yoshida and Miyakawa (1973) reported that the risk of urinary bladder neoplasms among dye applicators in Japan was 6.8 times the expected rate. Four of the specific benzidine-based dyes referred to in this report included Direct Black 38, Direct Green 1, Direct Red 17, and Direct Red 28. Genin (1977) in a U.S.S.R. study found an increased incidence of urinary bladder neoplasms in workers who dried or ground benzidine-based dyes.

Several recent studies have reported on the relationship between urinary bladder neoplasms and exposure to benzidine or its derived dyes. Xue-Yun *et al.* (1990) reported an increased risk of urinary bladder neoplasms in workers in Shanghai exposed to benzidine from 1946 to 1976, but no increased risk of bladder cancer in workers who used benzidine-derived dyes in 43 textile printing and dyeing factories. The authors suggest that the risk of bladder cancer in the occupational setting exists primarily in the presynthesis stage (where there is direct exposure to benzidine) but not in the postsynthesis stages where workers are exposed to dyes derived from benzidine.

The use of benzidine and other aromatic amines has been regulated in Japan since 1972, but because of the relatively long latency periods, urinary bladder neoplasms are still occurring in industrial cities. Shinka *et al.* (1991) evaluated dye workers in Wakayama City, Japan where an increase in urothelial neoplasms has been observed. They focused on early detection of urinary bladder neoplasms, and found that urinary cytology tests were responsible for the early detection of 55% of bladder cancers. The period from exposure to neoplasia ranged from 5 to 50 years, with a mean of 24 years. The peak incidence of neoplasia occurred 25 years after the peak benzidine production in 1955.

A study of all men employed from 1952 to 1985 by a chemical plant in New Jersey showed an increase in mortality from lung, liver, and bladder neoplasms in those workers involved in azo dye production. However, it was not possible to link the cancer with exposure to a particular chemical (Delzell *et al.*, 1989). Concerns over exposure to known bladder carcinogens including benzidine led Mason and Vogler (1989) to set up a screening program to monitor worker exposure to benzidine dyes and other chemicals in the New Jersey plant. These studies were also designed to detect early-stage bladder cancer. Meigs *et al.* (1986) reported on the exposure to benzidine and substituted benzidines (dichlorobenzidine, dianisidine, and diorthotoluidine) in a Connecticut plant that used and/or manufactured these chemicals from 1945 to 1965. They found an increased risk only for urinary bladder neoplasms.

Genetic Toxicity

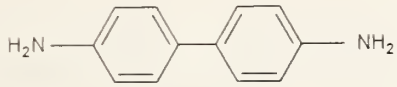
Results of short-term genotoxicity tests with C.I. Direct Blue 218 show no evidence of mutagenic activity. It was negative in standard *Salmonella* mutation assays which used strains TA98, TA100, TA1535, and TA1537 in a preincubation protocol, with and without liver S9 oxidative enzymes (Prival *et al.*, 1984; Mortelmans *et al.*, 1986), and in modified *Salmonella* tests with TA1538 which employed reductive metabolism (from flavin mononucleotide or rat cecal bacteria) in conjunction with S9 oxidative metabolism (Prival *et al.*, 1984; Reid *et al.*, 1984a). 3,3′-Dihydroxybenzidine, a putative metabolite of Direct Blue 218, was tested for mutagenicity in *Salmonella* strains TA98 and TA100 using standard preincubation protocol, with and without rat and hamster liver S9. It was mutagenic in TA98 and TA100 only in the presence of S9, and gave an equivocal response in TA100 without S9. In the Reid *et al.* (1984a) study, approximately 50% of the administered C.I. Direct Blue 218 was reduced by rat cecal bacteria, as determined by spectrophotometric analysis. However, the mutagenic potency of 3,3′-dihydroxybenzidine is insufficient for it to have been detected at the concentrations which would have resulted from partial reduction of the C.I. Direct Blue 218.

C.I. Direct Blue 218, administered by feeding or by injection, did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (Woodruff *et al.*, 1985), nor did it induce unscheduled DNA synthesis in hepatocytes of male Fischer 344 rats treated *in vitro* or *in vivo* (Mirsalis *et al.*, 1983).

Study Rationale

The National Toxicology Program's Benzidine Dye Initiative was established to determine the toxicity and carcinogenicity of representative dyes and congeners. The five chemical/dyes selected for *in vivo* toxicity and carcinogenicity testing are presented in Figure 1, and include 3,3′-dimethylbenzidine dihydrochloride (NTP, 1992a), 3,3′-dimethoxybenzidine dihydrochloride (NTP, 1990), C.I. Direct Blue 15 (NTP, 1992b), C.I. Acid Red 114 (NTP, 1991), and C.I. Direct Blue 218.

Long-term studies of 3,3′-dimethylbenzidine dihydrochloride and 3,3′-dimethoxybenzidine dihydrochloride were conducted in mice at the National Center for Toxicological Research (NCTR). Therefore, the NTP studies of 3,3′-dimethoxybenzidine dihydrochloride and 3,3′-dimethylbenzidine dihydrochloride were



Benzidine
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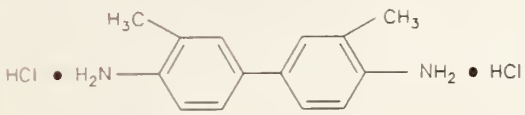

3,3′Dimethylbenzidine Dihydrochloride
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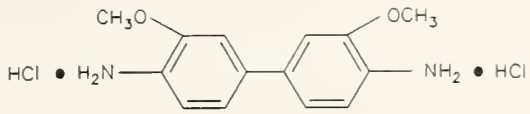
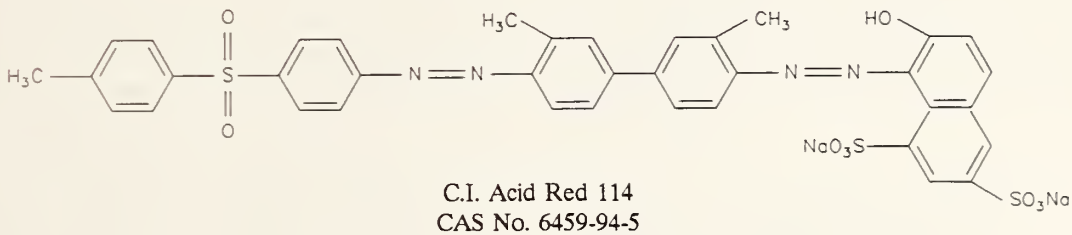
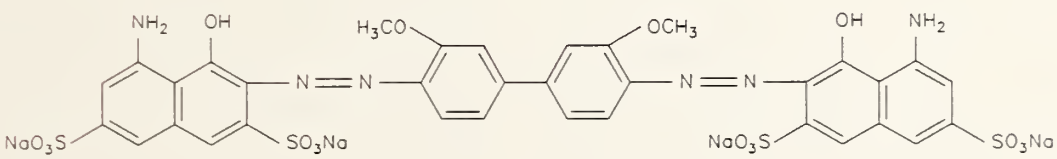


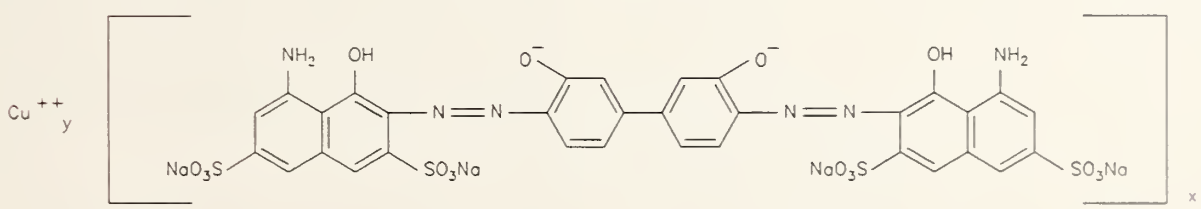
3,3′-Dimethoxybenzidine Dihydrochloride
CAS No. 20325-40-0



C.I. Acid Red 114
CAS No. 6459-94-5



C.I. Direct Blue 15
CAS No. 2429-74-5



C.I. Direct Blue 218
CAS No. 28407-37-6

FIGURE 1
Chemical Structure of Benzidine and Selected Benzidine Congeners and Dyes

conducted only in F344/N rats. Due to the limited stability of 3,3′-dimethoxybenzidine dihydrochloride, 3,3′-dimethylbenzidine dihydrochloride, C.I. Direct Blue 15, and C.I. Acid Red 114 in feed, these compounds were administered in the drinking water. C.I. Direct Blue 218, a copper chelated dye, was selected for study because of its widespread use and its non-mutagenic effects in standard tests of genotoxicity. Due to its limited solubility in water, administration was by feed to F344/N rats and B6C3F_1 mice.

MATERIALS AND METHODS

Procurement and Characterization of C.I. Direct Blue 218

C.I. Direct Blue 218 was obtained in two lots. Lot AT101681 was obtained from the Atlantic Chemical Company, Nutley, NJ, and desalted by Midwest Research Institute (Kansas City, MO). Lot F47238 was obtained from Ciba-Geigy Corporation and desalted by Atlantic Chemical Corporation. After lot AT101681 was desalted, it was assigned lot number M042382. The resultant salt content was 1.7% for lot M042382 and 0.7% for lot F47238. Assistance in obtaining the dyes was provided by Dyes Environmental and Toxicology Organization, Inc., Scarsdale, NY.

Identity and purity analyses were conducted on both lots at Midwest Research Institute. The study dye, a dark blue powder, was identified as C.I. Direct Blue 218 by infrared and ultraviolet/visible spectroscopy. Purity was determined by elemental analysis, weight loss on drying, azo group titrations, thin-layer chromatography, and high performance liquid chromatography (HPLC). Elemental analysis values could not be used to establish absolute purity or identity but were consistent with an organic to copper ratio of 1:2. Weight loss on drying for lots M042302 and F47238 indicated water content of 6% and 11%, respectively. Reduction titration of azo groups indicated purity of 90% and 83% for lots M042302 and F47238, respectively. The titration estimates of purity were probably enhanced by the presence of reducible low molecular weight organic impurities containing the azo group as well as inorganic copper salts. Comparison of the lots by HPLC showed no significant purity differences. The purity of the desalted lots was determined to be approximately 60%.

Thin layer chromatographic analysis resolved a major component and up to 13 impurities. As many as nine impurities with peak areas greater than 1% were detected by HPLC analysis, accounting for approximately 30% of the chromatographic peak area at a detection wavelength of 254 nm and 25% at a wavelength of 658 nm. No attempt was made to identify the chromatographic peaks. However, the concentrations of benzidine and 3,3′-dimethoxybenzidine dihydrochloride were determined. Benzidine could not be detected in either batch at levels greater than 1 ppm. 3,3′-Dimethoxybenzidine could not be detected in lot F47238 but was found at a level of 7 ppm in lot M042382.

The identity of the chemical was confirmed by infrared spectroscopy at the study laboratory. The stability of the bulk chemical was monitored using HPLC (major peak comparison to the reference standard) and visible spectrophotometry at approximately 650 nm. No degradation of the study material was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing C.I. Direct Blue 218 with feed (Table H1). Dose formulations were prepared prior to the initiation and at the midpoint of the 14-day studies, prior to the initiation and every 2 weeks for the 13-week studies, and every 2 weeks in the 2-year studies. Homogeneity was confirmed and the stability of the dose formulations was established for 3 weeks when stored in the dark at temperatures up to 25° C and for 1 week when stored open to air and light.

Periodic analyses of the dose formulations of C.I. Direct Blue 218 were conducted at the study laboratory and at the analytical chemistry laboratory using visible spectroscopy. For the 14-day studies, the dose formulations were analyzed prior to study initiation (Table H2). During the 13-week studies, the dose formulations were analyzed at the initiation, midpoint, and termination of the studies (Table H3). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks (Table H4). In the 2-year studies, 92 of 93 dose formulations analyzed were within 10% of the target concentration. Results of periodic referee analysis performed by the analytical chemistry laboratory were in good agreement with the results obtained by the study laboratory (Table H5).

14-Day Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Portage, MI). At receipt the rats were 28 to 35 days old and the mice were 35 to 42 days old. Rats and mice were quarantined for 15 days. At this time, two males and two females of each species were randomly selected and evaluated for evidence of disease. Groups of five male and five female rats and mice received 0, 1,000, 3,000, 7,000, 15,000, or 30,000 ppm C.I. Direct Blue 218 in feed for 14 days. Animals were housed five per cage. Water and feed were available *ad libitum* and feed consumption was measured once a week. Clinical findings were recorded once daily. Animals were weighed at study initiation, weekly, and prior to necropsy. A gross necropsy was performed on all rats and mice. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Complete histopathologic examinations were performed on 30,000 ppm animals; the liver of all rats and mice and the thymus and gallbladder of all mice were examined. Details of study design and animal maintenance are summarized in Table 1.

13-Week Studies

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to C.I. Direct Blue 218 and to determine the appropriate exposure levels to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). Upon receipt, the rats were 36 to 43 days old and the mice were 37 to 43 days old. The animals were quarantined for 14 (rats) or 13 (mice) days before exposure began. At this time, five rats and five mice of each sex were randomly selected and evaluated for evidence of disease. At the end of the studies, serologic analyses were performed on five rats and five mice of each sex using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice received 0, 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218 in feed for 13 weeks. Animals were housed five per cage. Water and feed were available *ad libitum,* and feed consumption was measured weekly. Clinical findings were recorded once daily. The animals were weighed at the beginning of the study, and weekly thereafter. Further details of study design and animal maintenance are summarized in Table 1.

At the end of the 13-week studies, blood was collected from the orbital sinus of all animals for clinical pathology analyses. The clinical pathology parameters measured are listed in Table 1. A necropsy was performed on all animals. The brain, heart, right kidney, liver, lungs, right testis, and thymus were weighed. Tissues for microscopic examination were embedded in paraffin, sectioned to a thickness of 4 to 6 μm and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all controls, animals killed moribund, and all 20,000 ppm animals. The liver of all rats and the testis and epididymis of all male rats, and the liver, gallbladder, and spleen of all mice were examined microscopically. Table 1 lists the tissues and organs routinely examined microscopically.

2-Year Studies

Study Design

Groups of 60 male and 60 female rats and mice received 0, 1,000, 3,000, or 10,000 ppm C.I. Direct Blue 218 in feed for 103 weeks. Ten rats and ten mice per group were designated for interim evaluations after 15 months of chemical administration.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA) for use in the 2-year studies. Rats were quarantined for 12 days, and mice were quarantined for 15 days before the beginning of the studies. Five rats and five mice of each sex were randomly selected and evaluated for evidence of disease. Serology samples were collected for viral screening. Rats and mice in the 2-year studies were 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats were housed five per cage, and mice were housed individually. Feed and water were available *ad libitum,* and feed consumption was measured once every 4 weeks (Appendix I). Racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

Animals were observed twice daily. Clinical findings were recorded weekly for the first 13 weeks, and monthly thereafter. Animals were weighed at the beginning of studies, weekly for the first 13 weeks, and every four weeks thereafter. At the 15-month interim evaluations blood was collected from the orbital sinus of all animals to determine hematology and clinical chemistry parameters and urine was collected from rats. The clinical pathology parameters measured are listed in Table 1. The brain, liver, right kidney, and spleen were weighed at the 15-month interim evaluations.

A complete necropsy was performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Histopathologic examinations were performed on all tissues with grossly visible lesions. Tissues examined are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist reviewed the forestomach, liver, and pharynx in male rats, the liver and uterus in female rats, the liver in male mice, the liver and ovary in female mice, and miscellaneous neoplasms in males and females of each species for accuracy and consistency of lesion diagnosis.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed selected slides of tissues when there was disagreement in diagnosis between the laboratory and quality assessment pathologists. The PWG reviewed all neoplasms of the pharynx and forestomach of rats; all pancreatic islet cell neoplasms of male rats; discrepancies in diagnosis of hepatocellular neoplasms in mice; all neoplasms of the forestomach, kidney, and intestine of mice; and miscellaneous neoplasms and nonneoplastic lesions. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of exposure levels or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses if they were found dead of other than natural causes or missing; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance included pairwise comparisons of each exposure group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, and urinalysis data which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Dunn (1964) and Shirley (1977). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunnett's or Dunn's test). Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are present in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff so that all discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of C.I. Direct Blue 218 was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, and *Salmonella typhimurium* strain TA1538 in rat cecal bacterial and flavin mononucleotide reduction systems, to induce chromosome damage in Chinese hamster ovary cells, and mutations in *Drosophila melanogaster*. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of C.I. Direct Blue 218 are part of a larger effort by NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure of the chemical and its responses in short-term *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al*, 1987; Zeiger *et al*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complimentarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of C.I. Direct Blue 218

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
International Research and Development Corporation (Mattawan, MI)	International Research and Developmental Corporation, (Mattawan, MI)	Microbiological Associates, Inc., (Bethesda, MD)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source		
Charles River Breeding Laboratories, Inc. (Portage, MI)	Simonsen Laboratories, Inc. (Gilroy, CA)	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Studies		
15 days	Rats: 14 days Mice: 13 days	Rats: 12 days Mice: 15 days
Average Age When Studies Began		
Rats: 6-7 weeks Mice: 7-8 weeks	Rats: 7-8 weeks Mice: 7-8 weeks	Rats: 6-7 weeks Mice: 7 weeks
Date of First Dose		
Rats: 7 July 1982 Mice: 30 June 1982	Rats: 21 December 1982 Mice: 20 December 1982	Rats: 15 May 1986 Mice: 13 August 1985
Duration of Dosing		
Rats: 14 days Mice: 14 days	Rats: 13 weeks Mice: 13 weeks	Rats: 105 weeks Mice: 105 weeks
Date of Last Dose		
Rats: 20 July 1982 Mice: 13 July 1982	Rats: 21 March 1983 Mice: 20 March 1983	Rats: 5 May 1988 Mice: 12 August 1987
Necropsy Dates		
Rats: 21 July 1982 Mice: 14 July 1982	Rats: 22 March 1983 Mice: 21 March 1983	Rats: 12-17 May 1988 Mice: 19-26 August 1987
Average Age at Necropsy		
Rats: 8-9 weeks Mice: 10 weeks	20-21 weeks	Rats: 110-112 weeks Mice: 112-113 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	60 males and 60 females
Method of Distribution		
Distributed using a table of random numbers.	Same as 14-day studies	Same as 14-day studies
Animals per Cage		
Rats: 5 Mice: 5	Rats: 5 Mice: 5	Rats: 5 Mice: 1

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of C.I. Direct Blue 218 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Method of Animal Identification		
Rats: Ear tag Mice: Toe clip	Rats: Ear tag Mice: Toe clip	Rats: Ear punch, toe clip Mice: Ear tag, toe clip
Diet		
NIH-07 open-formula mash diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 14-day studies	NIH-07 open formula powdered diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*
Maximum Storage Time for Feed		
120 days from milling date	Same as 14-day studies	Same as 14-day studies
Water		
Automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Cages		
Polycarbonate (Hazelton Systems, Inc., Aberdeen, MD or Lab Products, Inc., Maywood, NJ), changed twice weekly	Same as 14-day studies	Polycarbonate (Lab Products, Inc., Rochelle Park, NJ) changed once (mice) or twice (rats) weekly
Bedding		
Heat-treated hardwood chips (Northeastern Products, Corporation,Warrensburg, NY), changed twice weekly	Same as 14-day studies	Same as 14-day studies
Cage Filters		
Reemay spunbonded polyester (Snow Filtration, Cincinnati, OH), changed once every 2 weeks	Same as 14-day studies	Same as 14-day studies
Racks		
Stainless steel (Unifab Corporation, Kalamazoo, MI or Wahmann Manufacturing Co., Timonium, MO), changed once every 2 weeks	Same as 14-day studies	Stainless steel (Lab Products, Inc., Rochelle Park, NJ) change once every 2 weeks
Animal Room Environment		
Average temperature: Rats: 23° C Mice: 22° C Average relative humidity: Rats: 72% Mice: 68% Fluorescent light: 12 hours/day Room air changes: 12 changes/hour	Average temperature: Rats: 22° C Mice: 22° C Average relative humidity: Rats: 43% Mice: 49% Fluorescent light: 12 hours/day Room air changes: 13.5 changes/hour	Average temperature: Rats: 23° ± 1° C Mice: 22° ± 1° C Relative humidity: Rats: 50% ± 10% Mice: 56% ± 12% Fluorescent light: 12 hours/day Room air changes: 10 changes/hour

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of C.I. Direct Blue 218 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Doses		
0, 1,000, 3,000, 7,000, 15,000, or 30,000 ppm C.I. Direct Blue 218 in feed	0, 3,000, 10,000, or 20,000 ppm C.I. Direct Blue 218 in feed	0, 1,000, 3,000, or 10,000 ppm C.I. Direct Blue 218 in feed
Type and Frequency of Observation		
Observed twice daily; animal weighed initially, weekly and at the end of the studies; clinical findings recorded daily; feed consumption measured weekly.	Same as 14-day studies	Observed twice daily; animals weighed and clinical findings recorded weekly for 13 weeks, and monthly thereafter; feed consumption measured every 4 weeks.
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 14-day studies	Same as 14-day studies
Necropsy		
Necropsy performed on all animals. Organ weights recorded for brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals. Organ weights recorded for brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals. Organ weights recorded for brain, right kidney, liver, and spleen.
Clinical Pathology		
None	Blood samples were collected from the orbital sinus of all animals. ***Hematology:*** hematocrit, hemoglobin, erythrocytes, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, and leukocyte count and differential ***Clinical chemistry:*** blood urea nitrogen, alanine aminotransferase, and sorbitol dehydrogenase	Blood samples were collected from the orbital sinus of all animals at the 15-month interim evaluations. ***Hematology:*** hemoglobin, hematocrit, erythrocytes, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, platelets, reticulocytes, and leukocyte count and differential ***Clinical chemistry:*** blood urea nitrogen (rats), creatinine (rats), total protein (rats), albumin (rats), alkaline phosphatase (rats), alanine aminotransferase, creatinine kinase (rats), sorbitol dehydrogenase ***Urinalysis:*** total bile acids (rats)
Histopathology		
Complete histopathologic examinations were performed on all animals receiving 30,000 ppm. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), epididymis, esophagus, gallbladder (mice), large intestine (cecum, colon, rectum), (continued)	Complete histopathologic examinations were performed on all controls, all animals receiving 20,000 ppm, and all animals dying early. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), epididymis, esophagus, gallbladder (mice), large intestine (cecum, colon, rectum), (continued)	Complete histopathologic examinations were performed on all rats and mice. Adrenal gland, bone (including marrow), brain, clitoral gland, epididymis, esophagus gallbladder (mice), heart, kidney, large intestine (cecum, colon, rectum), liver, lung, mammary gland, mandibular lymph node, mesenteric lymph node, nose, ovary, pancreas, (continued)

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of C.I. Direct Blue 218 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Histopathology (continued) small intestine (duodenum, jejunum, ileum) heart, kidney, liver, lung, mammary gland, mandibular lymph node, mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, seminal vesicle, skin, spleen, sternebrae, stomach (forestomach and glandular), testes, thyroid gland, trachea, thymus, urinary bladder, and uterus. In addition, the liver of all rats and mice and the thymus and gall bladder of all mice were examined.	small intestine (duodenum, jejunum, ileum) heart, kidney, liver, lung, mammary gland, mandibular lymph node, mesenteric lymph node, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, seminal vesicle, skin, spleen, sternebrae, stomach (forestomach and glandular), testes, thyroid gland, trachea, thymus, urinary bladder, and uterus. In addition, the liver of all rats and mice, kidney of female rats, testis and epididymis of male rats, gallbladder of all mice, and spleen of mice receiving 3,000 and 10,000 ppm were examined.	parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

14-DAY STUDY

All male and female rats survived to the end of the study (Table 2). Male and female rats exposed to 30,000 ppm lost weight during the study and appeared emaciated during the last week of the study. Final mean body weights of other exposed groups of male and female rats were similar to those of the controls, although the mean body weight gain of male rats receiving 15,000 ppm was lower than that of the controls. Feed consumption by male and female rats that received 30,000 ppm was less than that by controls and feed spillage was observed at this exposure level, apparently as a result of reduced palatability of the diet.

Rats exposed to 30,000 ppm had a blue stain around the mouth, eye, and nose, and rats exposed to 15,000 and 30,000 ppm excreted blue feces. At necropsy 15,000 and 30,000 ppm rats had mild to moderate blue discoloration of the skin and intestine. Lower absolute organ weights in the 30,000 ppm groups were attributed to lower body weights. The relative heart weights of 30,000 ppm males and females and the relative thymus weight of 30,000 ppm males were significantly lower than those of the controls (Table F1).

Based on the lower final mean body weights observed in the 30,000 ppm groups, the high dose selected for the 13-week study was 20,000 ppm.

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 14-Day Feed Study of C.I. Direct Blue 218

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	198 ± 7	247 ± 8	49 ± 2		16.8	14.7
1,000	5/5	198 ± 8	248 ± 9	49 ± 3	100	18.0	14.3
3,000	5/5	197 ± 9	243 ± 9	46 ± 4	98	18.1	14.3
7,000	5/5	198 ± 8	240 ± 7	42 ± 3	97	16.6	14.1
15,000	5/5	201 ± 8	238 ± 7	37 ± 1*	96	17.1	16.8
30,000	5/5	196 ± 10	190 ± 12**	–6 ± 4**	77	12.2[d]	13.3[d]
Female							
0	5/5	138 ± 7	155 ± 7	16 ± 2		10.8	8.5
1,000	5/5	136 ± 6	157 ± 6	21 ± 2	102	11.0	10.1
3,000	5/5	139 ± 6	157 ± 6	18 ± 2	102	10.0	8.9
7,000	5/5	138 ± 6	156 ± 5	17 ± 1	101	10.7	8.4
15,000	5/5	141 ± 5	157 ± 5	16 ± 2	101	10.8	12.0
30,000	5/5	140 ± 4	139 ± 5	–1 ± 2**	90	8.1[d]	7.8[d]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Questionable values due to feed spillage

13-Week Study

All male and female rats survived to the end of the study (Table 3). The final mean body weights and the mean body weight gains of male and female rats receiving 20,000 ppm were significantly lower than those of the controls. The final mean body weight of male rats that received 20,000 ppm was 24% lower than that of the controls and the final mean body weight of female rats that received 20,000 ppm was 15% lower than that of the controls. The final mean body weights of males and females receiving 10,000 ppm were 4% lower than those of the controls. Feed consumption by rats in exposed groups was similar to that by the controls except in the 20,000 ppm groups, where feed spillage was noted. Dietary levels of 3,000, 10,000, and 20,000 ppm were estimated to deliver daily doses of approximately 200, 600, and 1,300 mg dye/kg body weight to males and 200, 800, and 1,400 mg/kg to females.

There were no clinical findings of toxicity. Blue discoloration of the fur and skin occurred in all exposed rats. Male and female rats receiving 10,000 and 20,000 ppm excreted blue feces.

The hematocrit, hemoglobin, mean erythrocyte volumes, and mean erythrocyte hemoglobin in male and female rats that received 10,000 and 20,000 ppm were significantly lower than those of controls (Table G1). These findings are indicative of a microcytic normochromic anemia and are suggestive of sequestration or a deficiency of iron, possibly secondary to extravascular hemolysis. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female rats receiving 20,000 ppm were significantly higher than those of controls, which is consistent with hepatocellular injury.

TABLE 3
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Feed Study of C.I. Direct Blue 218

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 1	Week 13
Male							
0	10/10	161 ± 5	334 ± 7	173 ± 6		14.4	16.4
3,000	10/10	161 ± 6	344 ± 4	183 ± 3	103	14.2	16.5
10,000	10/10	165 ± 7	322 ± 7	157 ± 6*	96	14.6	14.6
20,000	10/10	161 ± 7	255 ± 8**	93 ± 4**	76	12.8[d]	13.9[d]
Female							
0	10/10	119 ± 5	199 ± 4	81 ± 2		10.9	11.0
3,000	10/10	120 ± 4	205 ± 3	85 ± 3	103	10.3	12.0
10,000	10/10	118 ± 3	192 ± 2	74 ± 3	96	13.1	11.2
20,000	10/10	118 ± 3	169 ± 2**	51 ± 3**	85	10.7[d]	9.0[d]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Questionable values due to feed spillage

At necropsy, males and females that received 20,000 ppm had blue discoloration of the skin and subcutis, gastrointestinal tract, testis/epididymis, and uterus. Blue discoloration of the gastrointestinal tract was also observed in male and female rats receiving 10,000 ppm. The absolute and relative kidney weights of male and female rats exposed to 10,000 and 20,000 ppm were significantly greater than those of the controls (Table F2). The lower absolute liver, lung, thymus, and testis weights of 20,000 ppm rats were probably related to the lower body weights that occurred in these groups.

The principal histologic lesions associated with the administration of C.I. Direct Blue 218 for 13 weeks occurred in the liver of males and the liver and kidney of females (Table 4). The hepatic lesions occurred primarily in males and females receiving 20,000 ppm and in males receiving 10,000 ppm. The hepatic lesions were also slightly more frequent and severe in males than in females. The overall severity of the lesions was generally minimal.

The most consistent finding in the liver of male and female rats was the presence of macrophages,

TABLE 4
Incidences of Selected Lesions in Rats in the 13-Week Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	3,000	10,000	20,000
Male				
Liver[a]	10	10	10	10
Necrosis[b]	2 (1.5)[c]	0	0	9**(1.2)
Pigmented Macrophages	0	0	0	10**(1.5)
Multinucleated Hepatocytes[d]	0	0	6**(1.3)	7**(1.1)
Inflammation	2 (1.5)	0	2 (1.0)	6 (1.2)
Bile Duct Hyperplasia	0	0	0	5*(1.0)
Kidney	10	0	0	10
Pigment	0	–[e]	–	10**(1.5)
Testis	10	10	10	10
Degeneration	0	0	0	7**(2.9)
Females				
Liver	10	10	10	10
Necrosis	1 (1.0)	1 (2.0)	0	2 (1.0)
Pigmented Macrophages	0	0	0	9**(1.1)
Multinucleated Hepatocytes	0	0	0	0
Inflammation	1 (1.0)	0	1 (1.0)	2 (1.0)
Bile Duct Hyperplasia	0	0	0	3 (1.0)
Kidney	10	0	0	10
Microconcretions	7 (1.0)	–	–	9 (1.9)
Pigment	0	–	–	10**(1.8)

* Significantly different (P≤0.05) from the control group by the Fisher exact test
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average lesion severity for affected animals: 1 = minimal, 2 = mild, 3 = moderate
[d] Diagnosed as nuclear alteration by the study pathologist
[e] Organ not examined microscopically at this exposure level

presumably Kupffer cells, containing pale yellow-green intracytoplasmic pigment. The distribution of the pigmented macrophages was primarily periportal, but scattered pigmented macrophages were also randomly distributed along the sinusoids. The location and staining characteristics of the pigment are consistent with iron porphyrin-containing pigment possibly resulting from the lysis of erythrocytes. This finding is also consistent with the hematology results described above.

The principal lesions involving hepatocytes consisted of minimal individual cell necrosis and increased numbers of binucleated or multinucleated cells. The individual cell necrosis usually involved a single or, less frequently, small groups of hepatocytes scattered randomly throughout the liver lobules (Plate 1). Infrequently the necrotic cells were surrounded by a few mononuclear inflammatory cells. The binucleated and multinucleated hepatocytes were also randomly distributed within the lobules (Plate 2). The multinucleated cells were greatly enlarged and contained four to eight nuclei per cell. Minimal bile duct hyperplasia and periportal chronic inflammation were also observed in some 20,000 ppm male and female rats. The affected portal areas were generally infrequent and randomly distributed. They contained increased profiles of small ductules and infiltrates of mononuclear inflammatory cells.

The kidneys of male and female rats receiving 20,000 ppm also contained pale, yellow-green, slightly granular pigment within the cytoplasm of the proximal convoluted tubule epithelium. Hemoglobin resulting from intravascular hemolysis is known to be excreted by the renal glomerulus and absorbed by the proximal convoluted tubule epithelium. Microconcretions, consisting of intratubular, lamellated concretions of mineral at the corticomedullary junction (Plate 3) were observed in most control and 20,000 ppm female rats, but the number of microconcretions in the kidney sections were increased in females in the 20,000 ppm group. The mineral concretions were not associated with necrosis, inflammation, or other evidence of a host response. The distribution, histologic appearance, and sex difference observed in this study are similar to that reported to occur spontaneously.

Minimal to mild degeneration of the seminiferous epithelium was observed in the testes of seven of the 10 male rats receiving 20,000 ppm. The minimal lesions involved one or a few tubules while the mild lesions involved up to 25% of the tubules present. The affected seminiferous tubules exhibited decreased cellularity due to necrosis and loss of the germinal epithelium, and some contained small amounts of amorphous eosinophilic cellular debris or small concretions of mineral. Occasional multinucleated cells, representing fused spermatids, were seen in tubule lumens.

Dose selection rationale: Based on lower final mean body weights and the liver lesions observed in the 20,000 ppm groups, the high dose selected for the 2-year study was 10,000 ppm.

2-Year Study

Survival

Estimates of survival probabilities for male and female rats are presented in Table 5 and in the Kaplan-Meier curves in Figure 2. Survival of female rats receiving 10,000 ppm was slightly, but not significantly, lower than that of controls. Survival of males that received 10,000 ppm and males and females that received 1,000 or 3,000 ppm was similar to that of controls.

Table 5
Survival of Rats in the 2-Year Feed Study of C.I. Direct Blue 218

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	10	10	10	9
Natural deaths	7	3	5	9
Moribund	13	22	16	18
Animals surviving to study termination	30	25	29	24
Percent probability of survival at end of study[b]	60	50	58	48
Mean survival (days)[c]	638	652	655	631
Survival analysis[d]	P=0.285	P=0.670	P=1.000N	P=0.314
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	9	9	10	10
Natural deaths	4	7	6	9
Moribund	12	15	13	16
Animals surviving to study termination	35	29[e]	31	25
Percent probability of survival at end of study	70	57	62	51
Mean survival (days)	648	642	642	609
Survival analysis	P=0.086	P=0.326	P=0.615	P=0.069

[a] Censored from survival analyses; one high-dose male, one control female and one low-dose female designated for interim sacrifice and early and were not examined.
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A lower mortality in an exposure group is indicated by N.
[e] Includes one animal that died during the last week of the study.

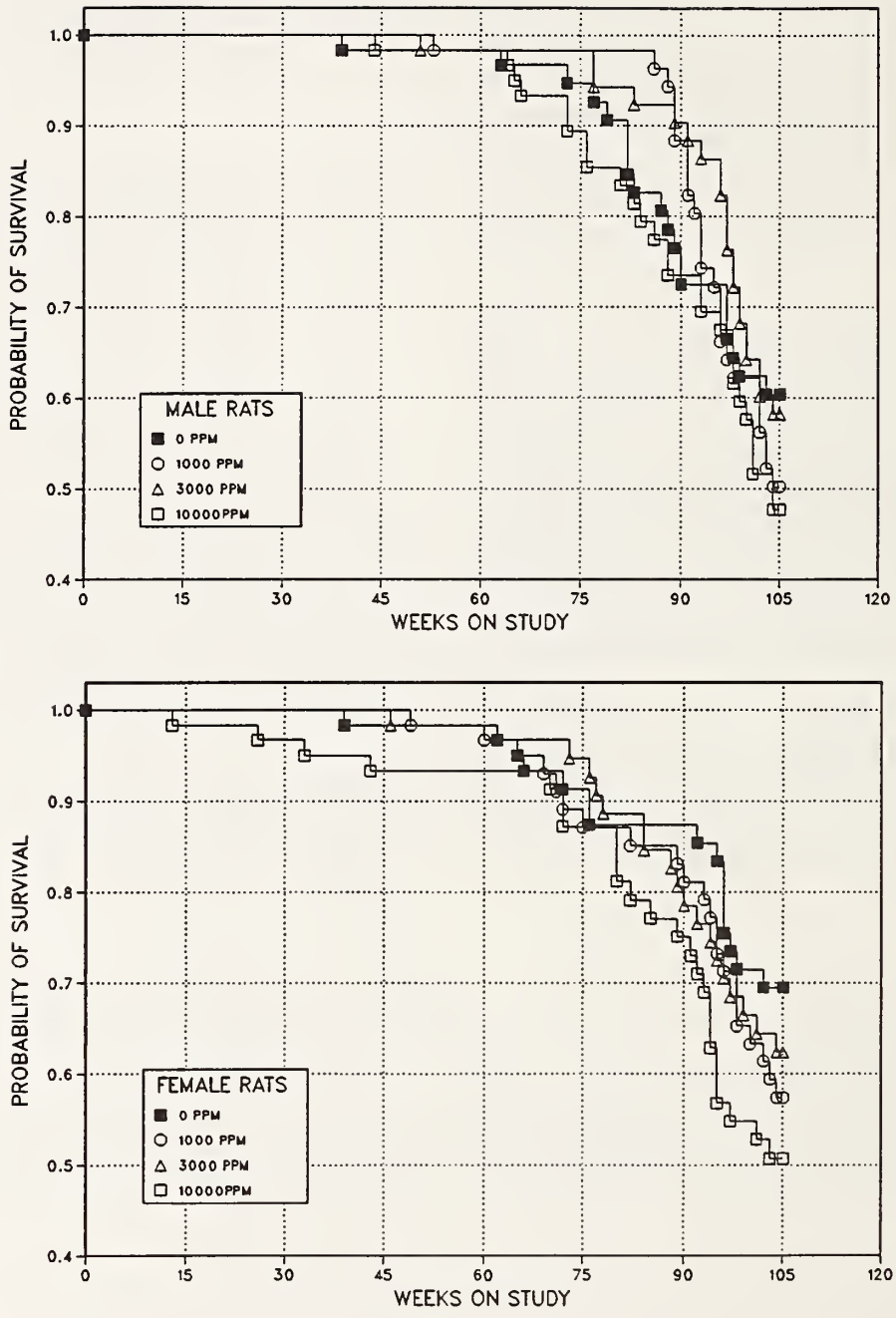


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Administered C.I. Direct Blue 218 in Feed for 2 Years

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of male and female rats in the 10,000 ppm groups were approximately 5% to 14% lower than controls after week 15, and the final mean body weights of male and female rats receiving 10,000 ppm were 11% and 9% lower than those of the controls (Tables 6 and 7 and Figure 3). Feed consumption by exposed male and female rats was similar to that by controls (Tables I1 and I2). Dietary levels of 1,000, 3,000, and 10,000 ppm were estimated to deliver daily doses of approximately 40, 120, and 440 mg dye/kg body weight to males and 50, 140, and 470 mg/kg to females (Tables I1 and I2). There were no clinical findings of toxicity attributable to the administration of C.I. Direct Blue 218.

Hematology and Clinical Chemistry

The neutrophil counts of males that received 3,000 and 10,000 ppm were significantly lower than those of the controls. The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values of females receiving 10,000 ppm were significantly lower than those of the controls. In males that received 10,000 ppm, only the mean erythrocyte hemoglobin value was significantly lower than that of the controls. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in males and females receiving 10,000 ppm were slightly but significantly higher than those of the controls (Table G2). While these hematology and clinical chemistry differences were considered chemical related, they were slight and not clinically important.

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218

Weeks on Study	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	119	60	120	100	60	122	103	60	123	103	60
2	156	60	154	98	60	156	100	60	154	99	60
3	190	60	189	99	60	192	101	60	190	100	60
4	217	60	217	100	60	221	101	60	216	99	60
5	237	60	231	98	60	233	99	60	234	99	60
6	257	60	250	97	60	256	100	60	251	98	60
7	273	60	271	99	60	274	100	60	268	98	60
8	287	60	285	99	60	290	101	60	283	98	60
9	302	60	300	100	60	305	101	60	298	99	60
10	304	60	312	103	60	318	105	60	311	102	60
11	325	60	324	100	60	329	101	60	320	99	60
12	334	60	332	99	60	337	101	60	327	98	60
13	343	60	340	99	60	346	101	60	336	98	60
17	379	60	364	96	60	378	100	60	360	95	60
21	399	60	388	97	60	397	100	60	371	93	60
25	411	60	405	98	60	407	99	60	378	92	60
29	425	60	422	99	60	433	102	60	401	94	60
33	436	60	432	99	60	444	102	60	409	94	60
37	446	60	440	99	60	448	100	60	410	92	60
41	452	59	444	98	60	453	100	60	408	90	60
45	456	59	449	98	60	450	99	60	415	91	59
49	466	59	454	97	60	462	99	60	419	90	59
53	465	59	454	98	60	462	99	59	420	90	59
57	467	59	454	97	59	458	98	59	415	89	59
61	461	59	447	97	59	456	99	59	414	90	59
65	466	58	454	97	59	456	98	59	413	89	58
69[a]	469	48	456	97	49	458	98	49	420	90	47
73	466	48	459	99	49	455	98	49	410	88	47
77	465	47	458	99	49	461	99	48	420	90	43
80	463	45	458	99	49	457	99	47	416	90	43
85	460	41	454	99	49	457	99	46	411	89	40
89	455	39	441	97	46	442	97	46	405	89	37
93	454	36	434	96	40	449	99	44	403	89	37
97	450	35	451	100	33	445	99	41	401	89	34
101	453	31	439	97	31	443	98	32	387	86	29
104	443	30	439	99	26	437	99	30	394	89	24
Mean for weeks											
1-13	257		256	100		260	101		255	99	
14-52	430		422	98		430	100		397	92	
53-104	460		450	98		453	98		409	89	

[a] Interim evaluation occurred during week 66.

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218

Weeks on Study	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	100	60	100	100	60	100	100	60	100	100	60
2	119	60	118	99	60	119	100	60	120	100	60
3	133	60	133	100	60	133	100	60	134	100	60
4	145	60	145	100	60	148	102	60	149	102	60
5	148	60	150	101	60	149	101	60	152	103	60
6	160	60	158	99	60	160	100	60	160	100	60
7	167	60	168	101	60	170	102	60	167	100	60
8	173	60	174	101	60	176	102	60	173	100	60
9	178	60	179	101	60	181	102	60	177	99	60
10	181	60	184	102	60	185	102	60	180	99	60
11	186	60	188	101	60	188	101	60	183	98	60
12	189	60	188	100	60	189	100	60	183	97	60
13	191	60	191	100	60	192	101	60	185	97	59
17	204	60	202	99	60	201	99	60	192	94	59
21	209	60	210	101	60	208	100	60	193	93	59
25	209	60	214	102	60	209	100	60	193	92	59
29	223	60	225	101	60	222	99	60	208	93	58
33	231	60	233	101	60	230	100	60	214	93	57
37	237	60	237	100	60	233	99	60	217	91	57
41	242	59	242	100	60	240	99	60	226	93	57
45	251	59	247	99	60	244	98	60	232	93	56
49	258	59	259	100	60	254	98	59	244	95	56
53	269	59	267	99	59	261	97	59	249	93	56
57	275	59	275	100	59	270	98	59	259	94	56
61	283	59	273	97	58	275	97	59	263	93	56
65	292	57	290	99	58	289	99	58	276	94	56
69[a]	304	47	306	101	47	305	100	48	288	95	46
73	314	46	311	99	45	310	99	48	294	94	43
77	322	44	318	99	44	317	98	46	302	94	43
80	332	44	329	99	44	327	99	44	310	94	40
85	336	44	334	99	43	334	100	42	312	93	39
89	334	44	331	99	43	333	100	40	308	92	38
93	337	43	336	100	41	332	99	38	309	92	35
97	341	38	342	100	36	337	99	35	316	93	28
101	346	36	340	98	32	338	98	33	310	90	27
104	346	35	340	98	30	339	98	32	314	91	25
Mean for weeks											
1-13	160		160	100		161	101		159	99	
14-52	229		230	100		227	99		213	93	
53-104	317		314	99		312	98		294	93	

[a] Interim evaluation occurred during week 66.

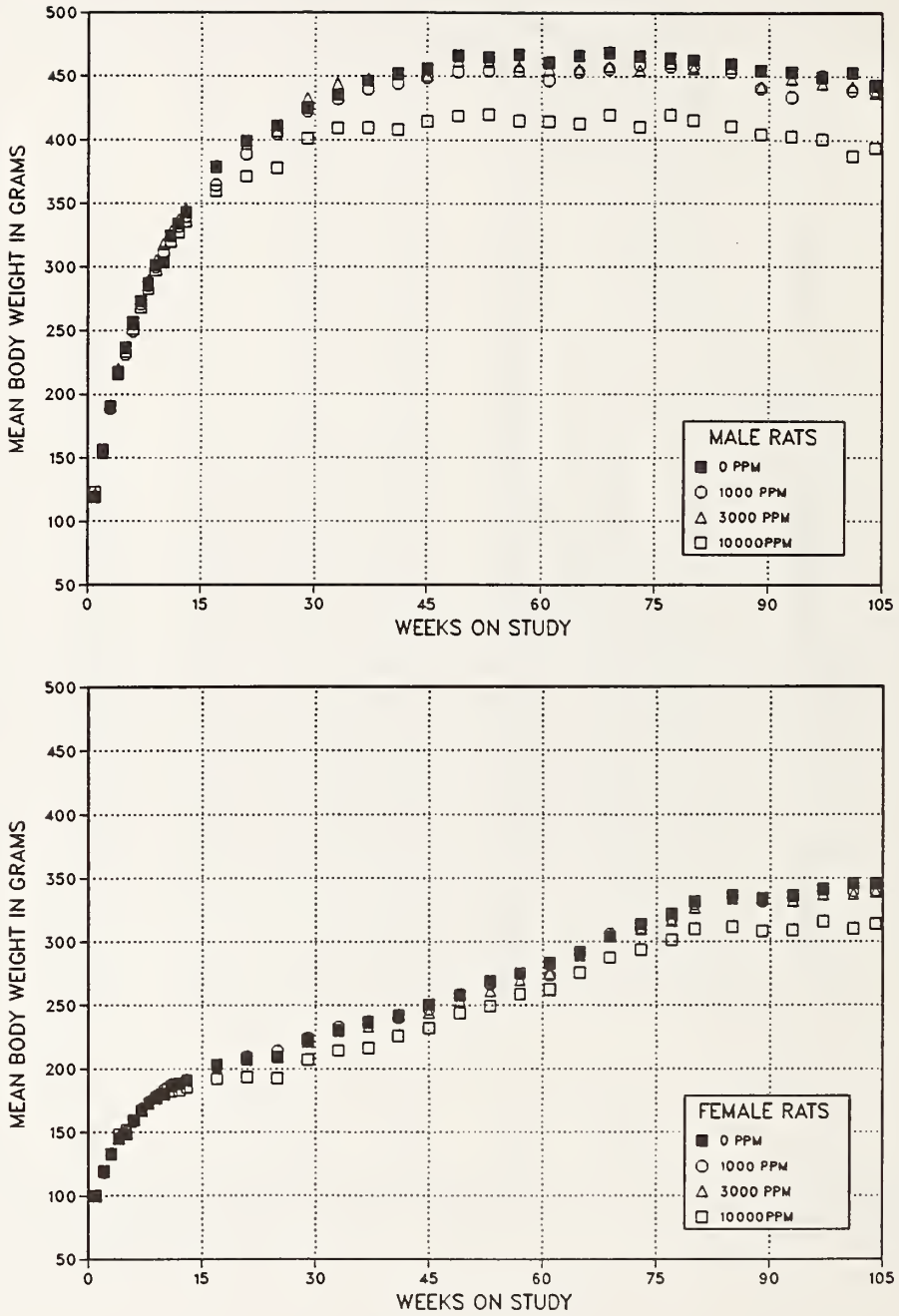


FIGURE 3
Growth Curves for Male and Female Rats Administered C.I. Direct Blue 218 in Feed for 2 Years

Pathology and Statistical Analysis of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the pharynx, forestomach, and uterus. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred at an incidence of at least 5% in at least one group, and the historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Pharynx: Squamous cell papilloma occurred with a significantly increased incidence in male rats that received 10,000 ppm (Tables 8 and A3) and exceeded the NTP historical incidence for this neoplasm in control male rats (10/1,253, 0.8%; range 0%-4%; Table A4a). The incidence of squamous cell papilloma in exposed female rats was not significantly increased (Tables 8 and B3). In addition, two male rats receiving 10,000 ppm had focal squamous epithelial hyperplasia of the mucosa in the posterior pharynx (Table A5). A squamous cell carcinoma occurred in one male rat that received 10,000 ppm, while another had a basosquamous tumor.

The squamous cell papillomas were exophytic, polypoid masses composed of fronds of well-differentiated, squamous epithelium supported by cores of fibrovascular stroma which occasionally formed a narrow base or stalk (Plate 4). The hyperplastic lesions of the posterior pharynx occurred as irregularly raised, focal areas of thickened squamous epithelium associated with mild hyperkeratosis and hypertrophy of the basal epithelial cells.

Histologically, the squamous cell carcinomas were exophytic papillary growths similar to the squamous cell papillomas. However, neoplastic cells focally invaded the fibrous stroma at the base of the squamous cell carcinoma. The basosquamous tumor was a well-demarcated lesion composed of irregular clusters of proliferating basal cells some of which exhibited focal squamous differentiation and keratin formation.

Forestomach: The incidences of basal cell hyperplasia in males receiving 3,000 or 10,000 ppm and in females receiving 10,000 ppm were significantly greater than those of the controls (Table 9). Basal cell hyperplasia was characterized by nodular downgrowths of small uniform cells with scant cytoplasm and round or oval hyperchromatic nuclei (Plate 5). While not significantly increased, the incidence of focal squamous hyperplasia occurred more frequently in the 3,000 and 10,000 ppm males than in the controls. Focal squamous hyperplasia of the forestomach was not observed in exposed or control female rats. Squamous cell papillomas were also observed in two males receiving 3,000 ppm and in one male and one female receiving 10,000 ppm. No papillomas were observed in control rats. The only malignant epithelial neoplasm of the forestomach, a squamous cell carcinoma, was seen in one 3,000 ppm male.

While the basal cell hyperplasia was clearly associated with the ingestion of C.I. Direct Blue 218, it is not certain if the other proliferative lesions were chemical related. Although the incidences of focal squamous hyperplasia, squamous cell papilloma, or squamous cell carcinoma were not significantly greater than those of the controls, they occurred only in rats receiving C.I. Direct Blue 218. Further, squamous cell neoplasms of the forestomach are relatively uncommon, and the incidence of three papillomas or carcinomas in the 3,000 ppm males exceeded the range in historical controls (4/1,253, 0.3%; range 0%-2%; Table A4b). Therefore, the low incidences of squamous hyperplasia or squamous cell papilloma in the exposed males may have been chemical related.

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Pharynx of Rats in the 2-Year Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	1,000	3,000	10,000
Male				
Pharynx[a]	50	50	50	50
Squamous Hyperplasia[b]	0	0	0	2
Squamous Cell Papilloma				
Overall rate[c]	0/50 (0%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted rate[d]	0.0%	0.0%	0.0%	19.3%
Terminal rate[e]	0/30 (0%)	0/25 (0%)	0/29 (0%)	4/24 (17%)
First incidence (days)	–[g]	–	–	684
Logistic regression test[f]	P<0.001	–	–	P=0.026
Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Squamous Cell Papilloma or Squamous Cell Carcinoma[h]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted rate	0.0%	0.0%	0.0%	23.3%
Terminal rate	0/30 (0%)	0/25 (0%)	0/29 (0%)	5/24% (21%)
First incidence (days)	–	–	–	684
Logistic regression test	P<0.001	–	–	P=0.013
Basosquamous Tumor Benign				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Female				
Pharynx				
Squamous Cell Papilloma[i]				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Logistic regression test	P=0.305	P=0.720	P=0.524N	P=0.490

[a] Number of animals necropsied
[b] Number of animals with lesion
[c] Number of neoplasm-bearing animals/number of animals necropsied
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A lower incidence in an exposure group is indicated by N.
[g] Not applicable; no neoplasms in animal group
[h] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 10/1,253 (0.8% ± 1.4%); range 0%-4%
[i] Historical incidence: 8/1,251 (0.6% ± 1.1%); range 0%-4%

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Rats in the 2-Year Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	1,000	3,000	10,000
Male				
Forestomach[a]	50	50	50	50
Basal Cell Hyperplasia[b]	0	2 (1.0)[c]	10**(1.0)	19**(1.4)
Squamous Cell Hyperplasia	1 (2.0)	1 (3.0)	6 (1.8)	4 (1.5)
Squamous Cell Papilloma				
Overall rate[d]	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
Logistic regression test[e]	P=0.337	–[f]	P=0.230	P=0.455
Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Squamous Cell Papilloma or Squamous Cell Carcinoma[g]				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Logistic regression test	P=0.370	–	P=0.114	P=0.455
Female				
Forestomach	50	49	50	49
Basal Cell Hyperplasia	1 (2.0)	1 (1.0)	5 (1.0)	11**(1.4)
Squamous Cell Papilloma[h]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)

** Significantly different (P≤0.05) from the control group by logistic regression
[a] Number of animals with forestomach examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Number of animals with neoplasm per number of animals with forestomach examined microscopically
[e] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal.
[f] Not applicable; no neoplasms in animal group
[g] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 4/1,253 (0.3% ± 0.8%); range 0%-2%
[h] Historical incidence: 2/1,251 (0.2% ± 0.6%); range 0%-2%

Uterus: The incidences of endometrial stromal polyps were significantly increased in exposed female groups (0 ppm, 1/50; 1,000 ppm, 12/50; 3,000 ppm, 10/50; 10,000 ppm, 10/50; Table B3). However, the control incidence was abnormally low, the incidences did not increase with dose, and the incidences were within the NTP historical range for this neoplasm (205/1,251, 16%; range 2%-30%; Table B4a). Histologically, the polyps were well vascularized, polypoid masses predominantly composed of loosely arranged stellate to spindle-shaped endometrial stromal cells and covered by cuboidal endometrial mucosal cells.

MICE

14-DAY STUDY

All mice survived to the end of the study (Table 10). Male and female mice exposed to 30,000 ppm lost weight, and appeared emaciated and hyperactive during the last week of the study. The final mean body weights of other exposed groups were similar to those of the controls. Feed consumption by exposed groups was similar to that by controls, except for the last week of the study, when the apparent amount of feed consumed by the 15,000 and 30,000 ppm groups was greater than that consumed by the controls. This increased feed consumption was related to increased feed spillage in these exposure groups.

Blue discoloration of the feces was observed in all exposed groups of male and female mice. At necropsy, the skin and gastrointestinal tracts of exposed male and female mice had blue discoloration. In male mice that received 15,000 ppm, the absolute and relative liver weights were significantly greater than those of the controls, and in males exposed to 30,000 ppm and females exposed to 15,000 and 30,000 ppm the relative liver weights were significantly greater than those of controls (Table F4). In the 30,000 ppm groups, there were some significantly lower absolute organ weights which were attributed to the lower body weights. The relative heart weight of 30,000 ppm females and the relative thymus weights of 30,000 ppm males and females were significantly lower than those of the controls (Table F4).

Based on the weight loss observed in the 30,000 ppm groups, the high dose selected for the 13-week study was 20,000 ppm.

TABLE 10
Survival, Mean Body Weights, and Feed Consumption of Mice in the 14-Day Feed Study of C.I. Direct Blue 218

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 1	Week 2
Male							
0	5/5	22.3 ± 0.9	24.0 ± 0.9	1.7 ± 0.1		2.4	2.6
1,000	5/5	22.3 ± 0.7	24.5 ± 0.9	2.2 ± 0.2	102	2.8	2.8
3,000	5/5	22.1 ± 0.7	24.2 ± 0.7	2.1 ± 0.2	101	2.6	2.5
7,000	5/5	22.0 ± 1.0	23.9 ± 0.8	1.9 ± 0.5	100	2.6	2.9
15,000	5/5	22.0 ± 0.9	22.3 ± 1.4	0.3 ± 0.6*	93	3.4	5.0[d]
30,000	5/5	22.1 ± 0.9	17.9 ± 0.8**	−4.2 ± 0.3**	75	4.0	5.6[d]
Female							
0	5/5	17.5 ± 0.4	18.8 ± 0.4	1.2 ± 0.2		2.2	1.8
1,000	5/5	17.4 ± 0.7	18.3 ± 0.5	0.9 ± 0.3	98	2.1	2.4
3,000	5/5	17.8 ± 0.4	18.8 ± 0.2	1.0 ± 0.3	100	2.5	2.2
7,000	5/5	17.6 ± 0.8	18.7 ± 0.8	1.1 ± 0.2	100	2.7	2.7
15,000	5/5	17.1 ± 0.4	18.0 ± 0.3	0.9 ± 0.4	96	3.1	3.9[d]
30,000	5/5	17.9 ± 1.0	15.1 ± 0.8**	−2.8 ± 0.4**	80	3.6	4.7[d]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Questionable values due to feed spillage

13-Week Study

There were no deaths attributed to the ingestion of C.I. Direct Blue 218 (Table 11). Two male mice, one receiving 3,000 ppm and the other 10,000 ppm, died during weeks 5 and 12, respectively. These deaths were attributed to fighting that took place among the group-housed males. All female mice survived to the end of the study. The final mean body weights and mean body weight gains of males and females that received 20,000 ppm and males that received 10,000 ppm were significantly lower than those of the controls. The final mean body weight of males that received 20,000 ppm was 24% lower than that of the controls and the final mean body weight of females receiving 20,000 ppm was 14% lower than that of the controls. Feed consumption by exposed mice was similar to that by the controls except in the 20,000 ppm groups, where feed spillage was noted and slightly higher levels of feed consumption were recorded. The dietary levels of 3,000, 10,000, and 20,000 ppm were estimated to deliver daily doses of approximately 400, 1,500, and 3,600 mg dye/kg body weight to males and 400, 1,800, and 4,000 mg/kg to females.

There were no clinical signs of toxicity. The fur, skin, and feces of all exposed groups of mice were discolored blue.

TABLE 11
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Feed Study of C.I. Direct Blue 218

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	23.7 ± 0.6	32.5 ± 0.9	8.8 ± 0.4		3.4	3.8
3,000	9/10[d]	23.6 ± 0.6	30.8 ± 0.7	7.7 ± 0.5	95	3.5	3.7
10,000	9/10[e]	23.4 ± 0.3	29.6 ± 0.2*	6.3 ± 0.3*	91	3.4	4.3[f]
20,000	10/10	23.7 ± 0.5	24.8 ± 1.1**	1.1 ± 1.2**	76	3.8[f]	4.9[f]
Female							
0	10/10	18.4 ± 0.4	25.5 ± 0.8	7.1 ± 0.7		3.1	3.5
3,000	10/10	18.6 ± 0.4	26.5 ± 0.8	7.9 ± 0.6	104	3.2	3.5
10,000	10/10	18.9 ± 0.4	24.2 ± 0.6	5.4 ± 0.3*	95	3.3	4.6[f]
20,000	10/10	18.5 ± 0.3	21.8 ± 0.3**	3.3 ± 0.5**	86	4.5[f]	4.4[f]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Week of death: 5
[e] Week of death: 12
[f] Questionable values due to feed spillage

The hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin values in males receiving 10,000 and 20,000 ppm were significantly lower than controls (Table G3). These findings are indicative of a microcytic normochromic anemia and are suggestive of sequestration or a deficiency of iron, possibly secondary to extravascular hemolysis. Mean erythrocyte volume and mean erythrocyte hemoglobin values in female mice exposed to 10,000 and 20,000 ppm were also significantly lower than those of the controls. Serum levels of alanine aminotransferase and sorbitol dehydrogenase in male and female mice receiving 10,000 and 20,000 ppm were significantly higher than those of controls (Table G3) and are consistent with the hepatic injury observed microscopically.

At necropsy, there was blue discoloration of the liver and kidneys of mice receiving 20,000 ppm. Most absolute and relative organ weight differences in the 20,000 ppm groups were attributed to lower body weights. However, despite the lower body weights of the 20,000 ppm groups, the absolute liver weights of mice in these groups were slightly greater and the relative liver weights were significantly greater than controls. This effect was attributed to the ingestion of C.I. Direct Blue 218.

The principal histologic lesions associated with the administration of C.I. Direct Blue 218 to mice occurred in the liver and spleen (Table 12). Liver lesions including centrilobular hepatocyte hypertrophy, karyomegaly, multifocal individual hepatocyte necrosis, and oval cell proliferation were observed in most mice receiving 20,000 ppm. The hepatic lesions in the 10,000 ppm groups were minimal and consisted only of hepatocyte hypertrophy and karyomegaly.

Hypertrophy of hepatocytes was primarily centrilobular in distribution, but in some mice the change was more extensive and involved midzonal and, infrequently, periportal hepatocytes as well. The affected hepatocytes were enlarged with an increased amount of eosinophilic cytoplasm and contained enlarged nuclei (karyomegaly) (Plate 6). Some nuclei were irregular rather than uniformly round and contained inclusions of invaginated cytoplasm. The individual cell necrosis was generally minimal in severity and involved a few individual hepatocytes or small groups of hepatocytes randomly distributed in the liver lobules similar to that observed in rats. The macrophages or Kupffer cells containing intracytoplasmic yellow-green pigment were observed in the periportal areas and to a lesser extent in the hepatic sinusoids. Unlike the rats receiving C.I. Direct Blue 218, proliferation of oval cells was observed in male and female mice. The oval cells radiated from the portal areas of the lobule, separating and distorting the periportal hepatic cords (Plate 7). The oval cells had scant cytoplasm and small, dense, oval nuclei.

While the spleens of all mice in each of the exposed and control groups contained hemosiderin-laden macrophages in the red pulp, the number of pigmented macrophages was increased in males and females receiving 20,000 ppm. The difference between the exposed and control groups was easily discernable, but generally slight. The increased accumulation of hemosiderin in the spleen of 20,000 ppm mice is consistent with an increased rate of destruction or lysis of erythrocytes and the hematology findings.

Dose selection rationale: Based on lower final mean body weights and on the liver lesions observed in the 20,000 ppm groups, the high dose selected for the 2-year study was 10,000 ppm.

TABLE 12
Incidences of Selected Lesions in Mice in the 13-Week Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	3,000	10,000	20,000
Male				
Liver[a]	10	10	10	10
Hepatocyte Hypertrophy[b]	0	0	5*(1.0)[c]	10**(2.1)
Karyomegaly	0	0	5*(1.0)	10**(1.9)
Necrosis	0	0	1 (1.0)	7**(1.0)
Oval Cell Hyperplasia	0	0	0	7**(1.6)
Pigmented Macrophages	0	0	1 (1.0)	10**(1.7)
Spleen	10	0	10	10
Pigmented Macrophages	10 (1.1)	–[d]	10 (1.9)	10 (2.1)
Female				
Liver	10	10	10	10
Hepatocyte Hypertrophy	0	0	10**(1.0)	10**(1.8)
Karyomegaly	0	0	10**(1.0)	10**(1.5)
Necrosis	0	0	0	5*(1.0)
Oval Cell Hyperplasia	0	0	0	7**(1.3)
Pigmented Macrophages	0	1 (1.0)	0	9**(1.0)
Spleen	10	0	10	10
Pigmented Macrophages	10 (1.1)	–	10 (2.0)	10 (2.1)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average lesion severity for affected animals: 1 = minimal, 2 = mild, 3 = moderate
[d] Organ not examined microscopically in these exposure groups

2-Year Study

Survival

Estimates of the survival probabilities for male and female mice are presented in Table 13 and in the Kaplan-Meier curves in Figure 4. Survival rates of exposed male and female mice were similar to those of the controls.

Body Weights, Feed Consumption, and Clinical Findings

The mean body weights of males and females receiving 10,000 ppm were 10% to 29% lower than those of controls during most of the study, while the mean body weights of 3,000 ppm males and females ranged from 1% to 10% lower than those of controls. The final mean body weights of 10,000 ppm males and females exposed were 19% and 27% lower than those of the controls (Tables 14 and 15 and Figure 5). Feed consumption by exposed mice was similar to that by controls (Tables I3 and I4). Dietary levels of 1,000, 3,000, and 10,000 ppm were estimated to deliver daily doses of approximately 120, 360, and 1,520 mg dye/kg body weight to males and 140, 470, and 2,050 mg/kg to females. There were no clinical signs of toxicity attributed to C.I. Direct Blue 218.

Table 13
Survival of Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Dose	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	9	10	10	10
Natural deaths	3	2	5	4
Moribund	2	2	3	1
Accidental deaths[a]	1	0	0	0
Missing[a]	1	0	0	0
Animals surviving to study termination	44	46	42	45
Percent probability of survival at end of study[b]	90	92	84	90
Mean survival (days)[c]	658	682	678	682
Survival analysis[d]	P=0.857	P=0.949N	P=0.602	P=1.000N
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	10	10	10	10
Natural deaths	4	2	2	5
Moribund	8	8	1	5
Accidental deaths[a]	0	0	0	1
Missing[a]	1	0	1	1
Animals surviving to study termination	37	40	46	38
Percent probability of survival at end of study	76	80	94	80
Mean survival (days)	638	673	682	648
Survival analysis	P=1.000N	P=0.695N	P=0.022N	P=0.792N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or lower mortality in an exposure group is indicated by N.

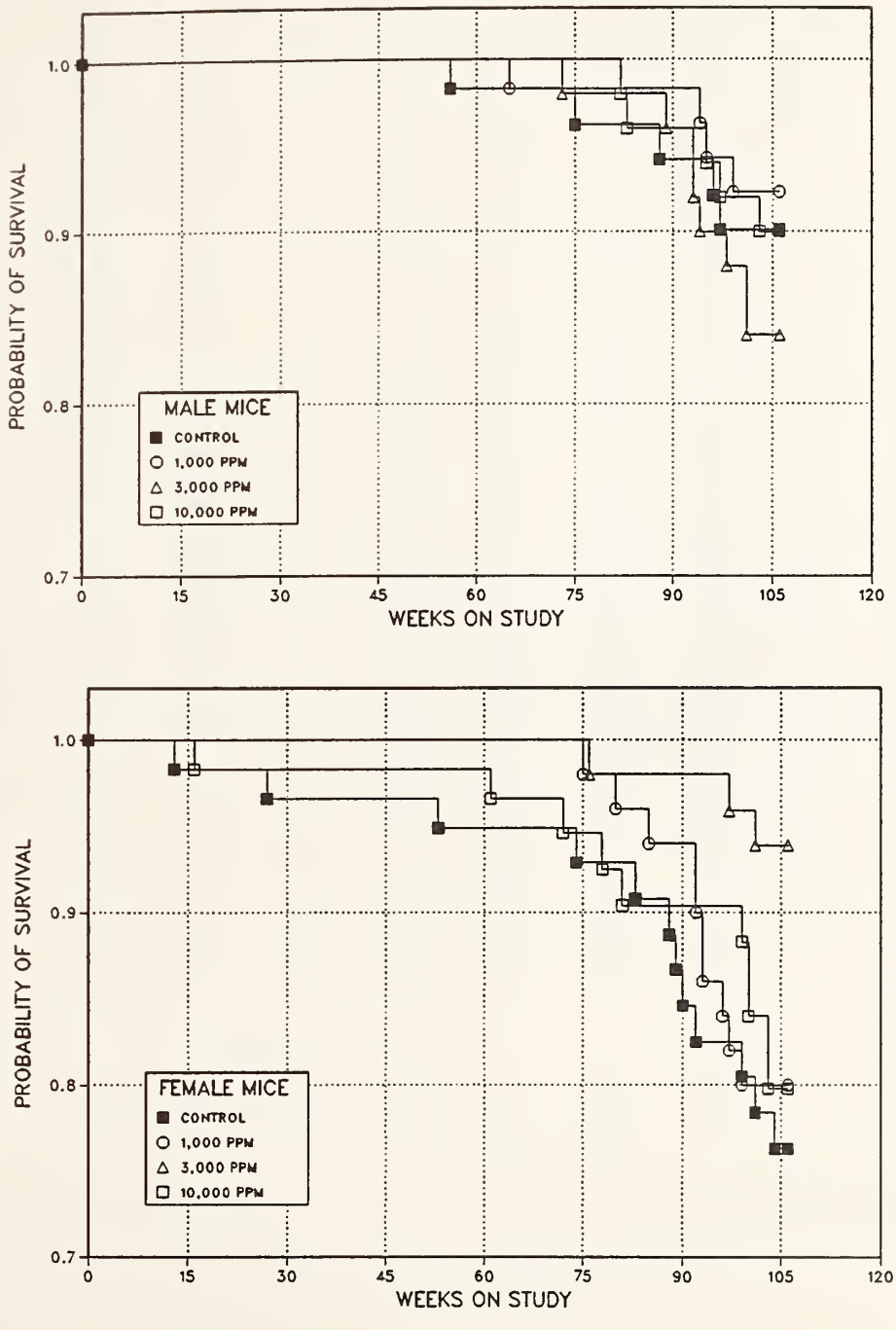


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered C.I. Direct Blue 218 in Feed for 2 Years

TABLE 14
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Weeks on Study	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.3	60	19.2	95	60	19.7	97	60	19.5	96	60
2	22.1	60	21.6	98	60	21.2	96	60	20.8	94	60
3	22.9	60	22.6	99	60	22.2	97	60	21.4	93	60
4	24.3	60	23.9	98	60	22.9	94	60	21.9	90	60
5	24.7	59	23.8	96	60	24.8	100	60	22.8	92	60
6	26.1	59	25.4	97	60	24.9	95	60	23.6	90	60
7	26.5	59	25.9	98	60	25.5	96	60	23.5	89	60
8	26.5	59	25.8	97	60	25.2	95	60	23.6	89	60
9	27.5	59	27.0	98	60	26.3	96	60	24.5	89	60
10	28.2	59	27.9	99	60	27.3	97	60	25.4	90	60
11	29.3	59	29.0	99	60	27.7	95	60	25.9	88	60
12	30.5	59	30.4	100	60	28.5	93	60	26.4	87	60
13	31.0	59	30.3	98	60	29.3	95	60	26.8	87	60
18	33.8	58	33.1	98	60	32.5	96	60	29.0	86	60
21	35.7	58	34.9	98	60	34.5	97	60	31.1	87	60
25	38.0	58	37.0	97	60	36.4	96	60	32.0	84	60
29	38.5	58	37.3	97	60	36.9	96	60	32.9	86	60
33	40.4	58	39.3	97	60	38.7	96	60	33.8	84	60
37	41.2	58	40.9	99	60	39.5	96	60	35.5	86	60
41	43.2	58	42.0	97	60	41.1	95	60	36.1	84	60
45	43.9	58	43.2	98	60	42.1	96	60	37.1	85	60
49	45.7	58	43.8	96	60	43.3	95	60	37.8	83	60
53	46.0	58	44.2	96	60	43.7	95	60	38.5	84	60
57	45.2	57	44.0	97	60	43.5	96	60	39.5	87	60
61	45.7	57	44.7	98	60	44.6	98	60	39.1	86	60
65	46.8	57	45.3	97	60	44.8	96	60	38.9	83	60
69[a]	46.7	48	45.7	98	49	44.4	95	50	38.6	83	50
73	47.0	48	46.3	99	49	44.2	94	50	38.2	81	50
77	46.9	47	46.7	100	49	45.2	96	49	38.6	82	50
81	47.2	47	46.4	98	49	45.3	96	49	38.3	81	50
85	47.8	47	46.1	96	49	44.8	94	49	38.2	80	48
89	44.8	46	45.0	100	49	43.9	98	49	36.9	82	48
93	44.9	46	44.0	98	49	43.3	96	48	36.3	81	48
97	43.5	45	43.2	99	47	42.1	97	45	35.1	81	47
101	41.3	44	40.6	98	46	40.3	98	43	33.5	81	46
105	41.4	44	39.7	96	46	40.8	99	42	33.5	81	45
Mean for weeks											
1-13	26.1		25.6	98		25.0	96		23.5	90	
14-52	40.0		39.1	98		38.3	96		33.9	85	
53-105	45.4		44.4	98		43.6	96		37.4	82	

[a] Interim evaluation occurred during week 65.

TABLE 15
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Weeks on Study	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.0	60	16.5	103	60	17.0	106	60	16.5	103	60
2	18.5	60	18.2	98	60	18.2	98	60	17.5	95	60
3	19.4	60	19.0	98	60	18.5	95	60	17.9	92	60
4	19.6	60	19.4	99	60	19.8	101	60	18.6	95	60
5	22.1	60	21.4	97	60	20.9	95	60	19.5	88	60
6	22.4	60	21.7	97	60	20.8	93	60	19.9	89	60
7	22.7	59	22.6	100	60	21.6	95	60	20.6	91	59
8	23.4	59	23.4	100	60	22.5	96	60	21.5	92	59
9	24.5	59	24.1	98	60	22.7	93	60	21.4	87	59
10	25.1	59	24.9	99	60	24.0	96	60	22.1	88	59
11	25.7	59	25.9	101	60	24.5	95	60	22.6	88	59
12	26.1	59	26.2	100	60	25.0	96	60	23.3	89	59
13	27.2	59	26.4	97	60	25.6	94	60	23.5	86	59
18	30.3	58	29.1	96	60	27.7	91	60	25.5	84	58
21	32.8	58	31.2	95	60	29.7	91	60	27.5	84	58
25	35.2	58	34.7	99	60	31.8	90	60	29.3	83	58
29	35.9	57	36.1	101	60	33.4	93	60	30.4	85	58
33	37.6	57	38.3	102	60	35.4	94	60	31.6	84	58
37	39.4	57	39.9	101	60	35.9	91	60	32.8	83	58
41	40.3	57	40.6	101	60	38.0	94	60	33.0	82	58
45	40.9	57	42.0	103	60	39.0	95	60	33.8	83	58
49	43.3	57	42.9	99	60	41.1	95	60	34.8	80	58
53	43.8	57	44.1	101	60	42.4	97	60	35.4	81	58
57	44.1	56	45.0	102	60	42.5	96	60	36.1	82	58
61	44.5	55	45.2	102	60	42.8	96	60	35.4	80	58
65	45.0	56	44.2	98	60	42.7	95	60	35.6	79	57
69[a]	46.5	46	45.6	98	50	44.2	95	49	35.4	76	47
73	47.2	46	45.4	96	50	44.1	93	49	35.3	75	46
77	47.2	45	46.4	98	49	44.8	95	48	35.4	75	46
81	46.1	45	45.9	100	48	44.4	96	48	34.6	75	44
85	46.4	44	46.1	99	47	44.2	95	48	34.4	74	43
89	44.5	42	44.9	101	47	43.2	97	48	33.6	76	43
93	44.8	40	43.3	97	45	42.9	96	48	32.6	73	43
97	43.6	40	42.6	98	42	40.6	93	48	30.9	71	43
101	40.3	38	40.3	100	40	38.2	95	47	28.6	71	40
105	40.0	37	39.7	99	40	37.8	95	46	29.0	73	38
Mean for weeks											
1-13	22.5		22.3	99		21.6	96		20.4	91	
14-52	37.3		37.2	100		34.7	93		31.0	83	
53-105	44.6		44.2	99		42.5	95		33.7	76	

[a] Interim evaluation occurred during week 65.

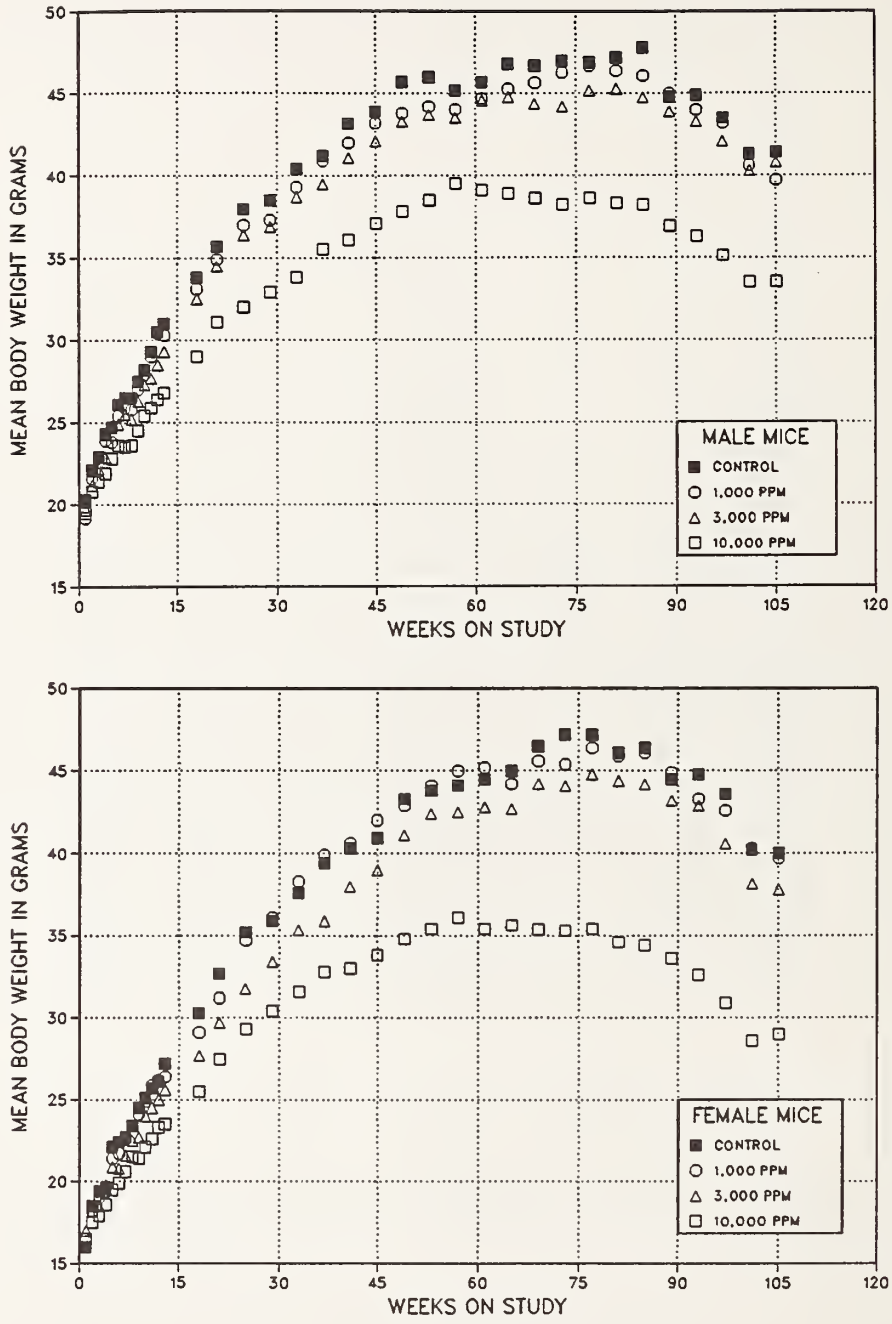


FIGURE 5
Growth Curves for Male and Female Mice Administered C.I. Direct Blue 218 in Feed for 2 Years

Hematology and Clinical Chemistry

Hematocrit, hemoglobin, and mean erythrocyte volumes in males and females that received 10,000 ppm were significantly lower than those of controls (Table G4). Although statistically significant, the decreases were slight and not clinically important. Serum levels of alanine aminotransferase and/or sorbitol dehydrogenase in male and female mice receiving 10,000 ppm were slightly but significantly higher than those of controls indicating hepatocellular damage.

Pathology and Statistical Analysis of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the liver, kidney, small intestine, and lung. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred at an incidence of at least 5% in at least one study group, and the historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: Despite the significantly lower body weights, the absolute liver weights of male and female mice receiving 10,000 ppm were similar to or slightly greater than those of the controls, and the relative liver weight of female mice receiving 10,000 ppm was significantly greater than that of controls at the 15-month interim evaluation (Table F6). This effect is consistent with findings in the 13-week study.

The incidences of hepatocellular foci (all types combined) in males and females receiving 10,000 ppm were significantly greater than those of controls at the 15-month interim evaluation (Tables 16, C5, and D5). Hepatocellular adenomas also occurred in exposed female mice (Tables 16 and D1). One hepatocellular carcinoma occurred in a male control mouse, and another in a female receiving 10,000 ppm.

In the 2-year study, the incidences of hepatocellular foci (all types combined) were slightly increased in males receiving 10,000 ppm and significantly increased in females receiving 10,000 ppm. Similarly, the incidences of hepatocellular adenoma were significantly increased in males that received 10,000 ppm and females that received 3,000 and 10,000 ppm (Tables 16, C3, and D3). There was a significantly increased incidence of hepatocellular carcinoma in male mice receiving 10,000 ppm. The incidence in females was also increased, but not significantly. The incidences of adenoma and carcinoma (combined) in the 10,000 ppm groups (males 90%; females 92%) far exceeded the range of historical incidences in untreated controls (Tables 16, C4, and D4).

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	1,000	3,000	10,000
Male				
15-Month Interim Evaluation				
Liver[a]	9	10	10	10
Basophilic Focus[b]	0	1	0	3
Mixed Cell Focus	0	1	0	0
Eosinophilic Focus	0	0	0	2
All Foci (combined)	0	1	0	5*
Hepatocellular Adenoma	0	0	0	1
Hepatocellular Carcinoma	1	0	0	0

(continued)

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

Dose (ppm)	0	1,000	3,000	10,000
Male (continued)				
2-Year Study				
Liver	50	50	50	50
Basophilic Focus	7	5	4	9
Eosinophilic Focus	13	12	10	28**
Mixed Cell Focus	3	5	2	1
All Foci (combined)	22	22	16	32
Hepatocellular Adenoma (Single or Multiple)				
Overall rate[c]	16/50 (32%)	19/50 (38%)	17/50 (34%)	40/50 (80%)
Adjusted rate[d]	34.7%	40.4%	38.6%	81.6%
Terminal rate[e]	14/44 (32%)	18/46 (39%)	15/42 (36%)	36/45 (80%)
First incidence (days)	519	689	686	576
Logistic regression test[f]	P<0.001	P=0.371	P=0.526	P<0.001
Multiple Hepatocellular Adenoma				
Overall rate	4/50 (8%)	10/50 (20%)	10/50 (20%)	31/50 (62%)
Hepatocellular Carcinoma				
Overall rate	7/50 (14%)	3/50 (6%)	8/50 (16%)	17/50 (34%)
Adjusted rate	15.9%	6.5%	17.1%	36.0%
Terminal rate	7/44 (16%)	3/46 (7%)	4/42 (10%)	15/45 (33%)
First incidence (days)	737 (T)	737 (T)	622	568
Logistic regression test	P<0.001	P=0.141N	P=0.506	P=0.019
Hepatocellular Adenoma or Carcinoma[g]				
Overall rate	21/50 (42%)	20/50 (40%)	23/50 (46%)	45/50 (90%)
Adjusted rate	45.5%	42.6%	48.8%	90.0%
Terminal rate	19/44 (43%)	19/46 (41%)	18/42 (43%)	40/45 (89%)
First incidence (days)	519	689	622	568
Logistic regression test	P<0.001	P=0.451N	P=0.446	P<0.001
Female				
15-Month Interim Evaluation				
Liver	10	10	10	10
Eosinophilic Focus	0	0	0	6**
Clear Cell Focus	0	0	2	1
Basophilic Focus	0	0	0	1
All Foci (combined)	0	1	2	8**
Hepatocellular Adenoma	0	1	1	1
Hepatocellular Carcinoma	0	0	0	1

(continued)

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver of Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

Dose (ppm)	0	1,000	3,000	10,000
Female (continued)				
2-Year Study				
Liver	49	50	49	49
Basophilic Focus	4	2	6	7
Clear Cell Focus	2	2	1	12**
Eosinophilic Focus	11	7	11	21*
Mixed Cell Focus	1	1	0	0
All Foci (combined)	13	11	17	29**
Hepatocellular Adenoma (Single or Multiple)				
Overall rate	7/49 (14%)	12/50 (24%)	17/49 (35%)	41/49 (84%)
Adjusted rate	18.4%	30.0%	36.1%	91.1%
Terminal rate	6/37 (16%)	12/40 (30%)	16/46 (35%)	34/38 (89%)
First incidence (days)	701	739 (T)	676	541
Logistic regression test	P<0.001	P=0.185	P=0.041	P<0.001
Multiple Hepatocellular Adenoma				
Overall rate	1/49 (2%)	4/50 (8%)	8/49 (16%)	35/49 (71%)
Hepatocellular Carcinoma				
Overall rate	5/49 (10%)	5/50 (10%)	6/49 (12%)	12/49 (24%)
Adjusted rate	13.5%	12.0%	13.0%	30.6%
Terminal rate	5/37 (14%)	4/40 (10%)	6/46 (13%)	11/38 (29%)
First incidence (days)	739 (T)	648	739 (T)	689
Logistic regression test	P=0.012	P=0.586N	P=0.603N	P=0.061
Hepatocellular Adenoma or Carcinoma[h]				
Overall rate	10/49 (20%)	15/50 (30%)	21/49 (43%)	45/49 (92%)
Adjusted rate	26.3%	36.4%	44.7%	100.0%
Terminal rate	9/37 (24%)	14/40 (35%)	20/46 (43%)	38/38 (100%)
First incidence (days)	701	648	676	541
Logistic regression test	P<0.001	P=0.226	P=0.045	P<0.001

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the control group by the Fisher exact test (15-month interim) or the logistic regression test (2-year study)
** P≤0.01
[a] Number of animals with liver examined microscopically
[b] Number of animals with lesion
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A lower incidence in an exposure group is indicated by N.
[g] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 485/1,366 (35.5% ± 14.3%); range 10%-68%
[h] Historical incidence: 223/1,363 (16.4% ± 10.7%); range 3%-42%

Kidney: Four renal tubule adenomas occurred in exposed males (Tables 17 and C1). One renal tubule carcinoma occurred in a 1,000 ppm male. However, the incidences were not statistically significant or dose related. No more than one renal tubule neoplasm has been seen in male historical control groups from recent NTP studies (4/1,366, 0.3%; Table C4b). No kidney neoplasms were seen in females.

TABLE 17
Incidences of Neoplasms of the Kidney of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	1,000	3,000	10,000
Renal Tubule Adenoma				
Overall rate[a]	0/50 (0%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate[b]	0.0%	4.3%	2.4%	2.2%
Terminal rate[c]	0/44 (0%)	2/46 (4%)	1/42 (2%)	1/45 (2%)
First incidence (days)	–[e]	737 (T)	737 (T)	737 (T)
Logistic regression test[d]	P=0.628	P=0.248	P=0.491	P=0.504
Renal Tubule Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Renal Tubule Adenoma or Carcinoma[f]				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	0.0%	6.5%	2.4%	2.2%
Terminal rate	0/44 (0%)	3/46 (7%)	1/42 (2%)	1/45 (2%)
First incidence (days)	–	737 (T)	737 (T)	737 (T)
Logistic regression test	P=0.588N	P=0.129	P=0.491	P=0.504

(T) Terminal sacrifice
[a] Number of animals with neoplasm per number of animals with kidney examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence in animals surviving until the end of the study
[d] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A negative trend is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 4/1,366 (0.3% ± 0.7%); range 0%-2%

Small intestine: Carcinomas of the small intestine were observed in one male mouse receiving 10,000 ppm at the 15-month interim evaluation and in three other males receiving 10,000 ppm at the end of the 2-year study (Tables 18 and C1). A carcinoma of the small intestine was also seen in one control male and an adenoma was seen in one male that received 1,000 ppm. While the incidence of small intestine neoplasms in the 10,000 ppm group was low and not significantly greater than that of controls, small intestine neoplasms are rare in untreated control male mice (12/1,374, 0.9%; range 0%-4%; Table C4c). Because the incidence in the 10,000 ppm males exceeded the upper range of historical controls, the carcinomas of the small intestine may have been chemical related.

Carcinomas of the small intestine were also seen in one female mouse receiving 3,000 ppm and one female mouse receiving 10,000 ppm (Tables 18 and D1). The incidence of neoplasms of the small intestine in untreated historical control female mice is 10/1,371 (0.7%; range 0%-6%; Table D4b). Thus, the incidence in each of the exposed groups was well within the range of historical controls.

Lung: There was a significant negative trend in the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in male mice, and the incidence in the 10,000 ppm group was significantly less than that of the controls (Table C3). While the incidence of alveolar/bronchiolar neoplasms in female mice receiving 10,000 ppm was also decreased, the decrease was not statistically significant (Table D3).

Genetic Toxicology

C.I. Direct Blue 218 (33 to 10,000 μg/plate) did not induce mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the standard NTP assay which used a preincubation protocol, with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 oxidative enzymes (Table E1; Mortelmans *et al.*, 1986). It was also tested in a modified *Salmonella* test protocol which employed reductive metabolism supplied by flavin mononucleotide or rat cecal bacteria, followed by oxidative metabolism; results of this test, using strain TA1538, were also negative (Table E2; Reid *et al.*, 1984a).

In cytogenetic tests with cultured Chinese hamster ovary cells, C.I. Direct Blue 218 induced a small but significant increase in sister chromatid exchanges at the highest dose tested (200 μg/mL) in the second of two trials conducted without S9 (Table E3); no increase in sister chromatid exchanges was observed in either of two trials conducted with S9. No increase in chromosomal aberrations was seen in cultured Chinese hamster ovary cells treated with C.I. Direct Blue 218, at concentrations up to 500 μg/mL, with and without S9 (Table E4).

No increase in the frequency of sex-linked recessive lethal mutations was observed in the offspring of male *Drosophila melanogaster* administered C.I. Direct Blue 218 by feeding (10,000 ppm) or by injection (1,000 ppm) (Table E5; Woodruff *et al.*, 1985).

TABLE 18
Incidences of Neoplasms of the Small Intestine of Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Dose (ppm)	0	1,000	3,000	10,000
Male				
15-Month Interim Evaluation				
Small Intestine: Carcinoma				
Overall rate[a]	0/9 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)
2-Year Study				
Small Intestine: Adenoma[b]				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Small Intestine: Carcinoma[c]				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[d]	2.3%	0.0%	0.0%	6.7%
Terminal rate[e]	1/44 (2%)	0/46 (0%)	0/42 (0%)	3/45 (7%)
First incidence (days)	737 (T)	–[g]	–	737 (T)
Logistic regression test[f]	P=0.055	P=0.491N	P=0.509N	P=0.314
Female				
2-Year Study				
Small Intestine: Carcinoma[h]				
Overall rate	0/49 (0%)	0/50 (0%)	1/49 (2%)	1/49 (2%)

(T) Terminal sacrifice
[a] Number of animals with neoplasm per number of animals necropsied
[b] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 5/1,374 (0.4% ± 1.0%); range 0%-4%
[c] Historical incidence: 7/1,374 (0.5% ± 1.0%); range 0%-4%
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A lower incidence in an exposure group is indicated N.
[g] Not applicable; no neoplasms in animal group
[h] Historical incidence: 8/1,371 (0.6% ± 1.0%); range 0%-6%

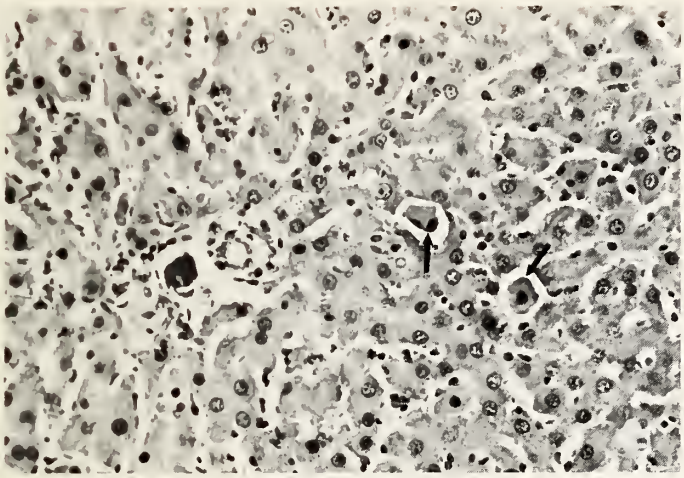


PLATE 1
Liver of a male F344/N rat exposed to 20,000 ppm C.I. Direct Blue 218 in feed for 13 weeks. Individual necrotic hepatocytes (arrows) have dark, pyknotic nuclei. H&E, 80×.

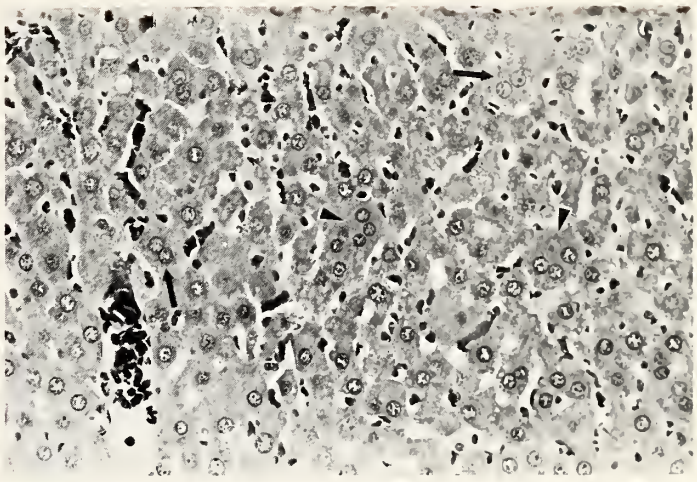


PLATE 2
Liver of a male F344/N rat exposed to 20,000 ppm C.I. Direct Blue 218 in feed for 13 weeks. Note binucleate (arrows) and multinucleate hepatocytes (arrowheads). H&E, 80×.

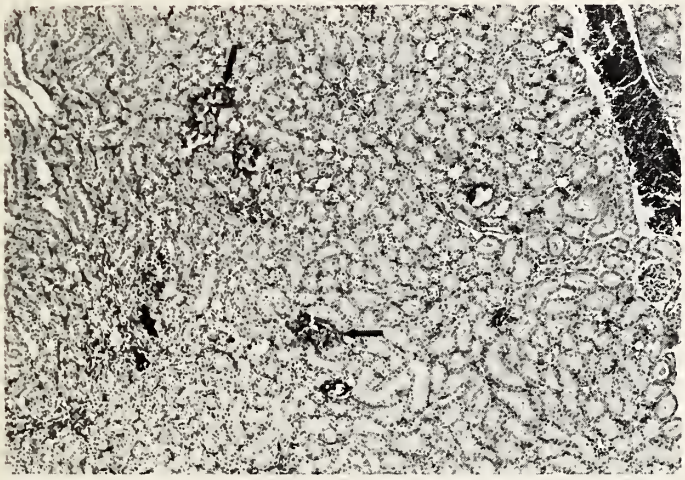


PLATE 3
Kidney of a female F344/N rat exposed to 20,000 ppm C.I. Direct Blue 218 in feed for 13 weeks. Renal tubules along the corticomedullary junction contain microconcretions of mineral (arrows). H&E, 20×

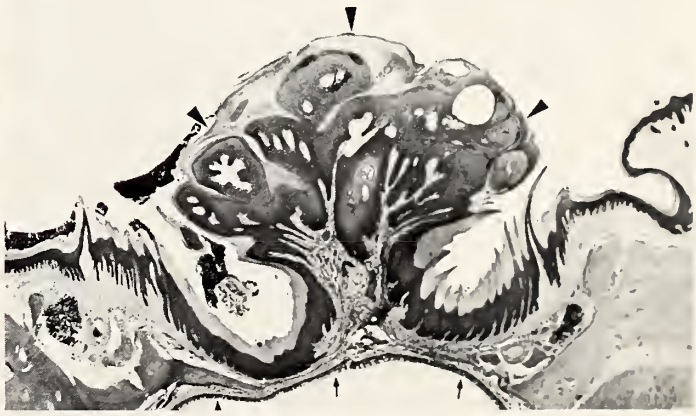


PLATE 4
Squamous cell papilloma (arrowheads) in the posterior oral cavity (pharynx) of a male F344/N rat exposed to 10,000 ppm C.I. Direct Blue 218 in feed for 2 years. The exophytic polypoid mass of squamous epithelium is supported by fibrous stalk. Arrows indicate the respiratory epithelium lining the nasopharynx. H&E, 8×.

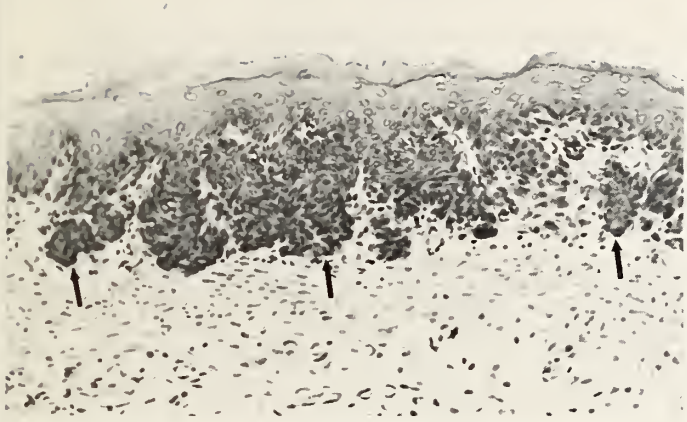


PLATE 5
Basal cell hyperplasia in the forestomach of a male F344/N rat exposed to 10,000 ppm C.I. Direct Blue 218 in feed for 2 years. Note the nodular downgrowths of small, uniform basal cells (arrows) into the adjacent submucosa. H&E, 50×.

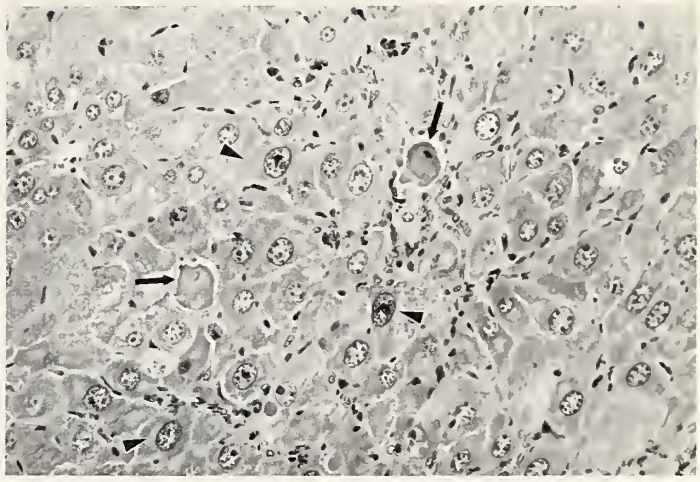


PLATE 6
Liver of a B6C3F$_1$ mouse exposed to 20,000 ppm C.I. Direct Blue 218 in feed for 13 weeks. Note individual hepatocyte necrosis (arrows) and enlarged (hypertrophic) hepatocytes (arrowheads) with enlarged nuclei (karyomegaly). H&E, 80×.

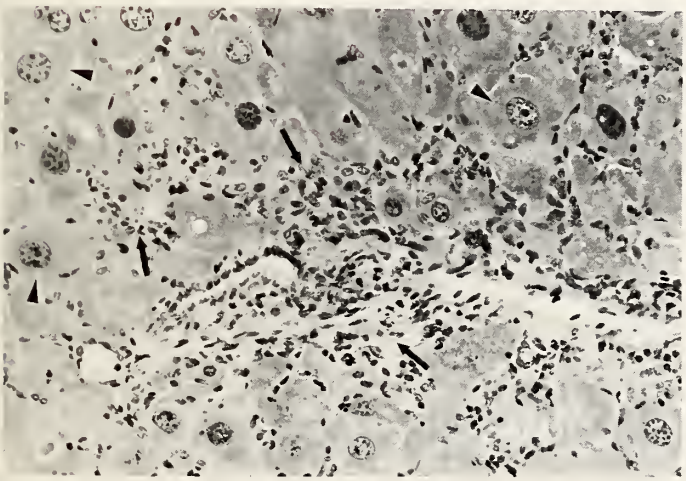


PLATE 7
Liver of a B6C3F$_1$ mouse exposed to 20,000 ppm C.I. Direct Blue 218 in feed for 13 weeks. Note hypertrophic hepatocytes (arrowheads) and proliferation of cells with small oval nuclei (oval cell proliferation) radiating from periportal area (arrows). H&E, 80×.

DISCUSSION AND CONCLUSIONS

The Benzidine Dye Initiative was established in cooperation with NIEHS, NCTR, NIOSH, USEPA, and the Consumer Product Safety Commission (NIOSH, 1980, 1983) to identify the toxic and carcinogenic properties of representative chemicals/dyes used in the workplace. Since there are more than 100 benzidine-based dyes, the initiative focused on studying the toxicity and carcinogenicity of five representative benzidine dyes/congeners: C.I. Acid Red 114 and its parent congener 3,3′-dimethylbenzidine dihydrochloride; C.I. Direct Blue 15 and its parent congener 3,3′-dimethoxybenzidine dihydrochloride; and the copper chelated dye, C.I. Direct Blue 218 (congener 3,3′-dihydroxybenzidine). The first four chemicals/dyes are unstable in feed and were administered in the drinking water to F344/N rats in long-term toxicity and carcinogenicity studies (NTP, 1990; 1991; 1992a,b).

C.I. Direct Blue 218 was the one dye not mutagenic in the *Salmonella* reductive metabolism test system (Tables 19 and 20) and was tested to determine if its spectrum of carcinogenicity differed from those of the other four mutagenic chemicals/dyes. The C.I. Direct Blue 218 used in these studies was representative of the dye in commercial use and contained approximately 60% of the major component. The dye was desalted prior to use to reduce the salt content from approximately 17% to 3%. C.I. Direct Blue 218 has limited solubility in water, therefore the dose formulations for these studies were prepared by mixing appropriate amounts of C.I. Direct Blue 218 in feed.

TABLE 19
Comparison of National Toxicology Program Mutagenicity Tests Results for Selected Benzidine Dyes[a]

Chemical Name	*Salmonella*[b]	CHO SCE	CHO Abs	*Drosophila* SLRL	*Drosophila* RT
Benzidine	+[c]	+	+	NT	NT
3,3′-Dimethoxybenzidine	+[c]	+	+	–	NT
3,3′-Dimethylbenzidine	+[c]	+	+	+	–
C.I. Acid Red 114	+[c]	–	–	–	NT
C.I. Direct Blue 218	–	+w	–	–	NT
C.I. Direct Blue 15	+	–	–	NT	NT

[a] CHO SCE = Chinese hamster ovary cell sister chromatid exchange test; CHO Abs = Chinese hamster ovary cell chromosomal aberration test; SLRL = sex-linked recessive lethal test; RT = reciprocal translocation test; + = positive; – = negative; +w = weak evidence for positive response; NT = not tested. For details of experimental technique, see Appendix E.

[b] Results are for strain TA1538, tested with a reductive metabolism protocol which allows for *in vitro* reduction of the azo linkages, mimicking the metabolism of these compounds in the human intestinal tract, and release of the parent amine, which can then be oxidatively metabolized using an induced rat or hamster liver S9 system (Reid *et al.*, 1984a).

[c] These compounds were also mutagenic when tested in a standard *Salmonella typhimurium* preincubation protocol employing oxidative metabolism only.

TABLE 20
Summary of *Salmonella* Mutagenicity Studies with the Selected Benzidine Congeners and Congener Dyes[a]

Congener/Dye	% Purity[c]	Metabolic Activation System[b]			
		None	S9	FMN	Cecal
Benzidine	>99	–	+	+	+
C.I. Direct Black 38	66	+	+	+	+
C.I. Direct Blue 2	46	+	+	+	NT
C.I. Direct Blue 6	60	–	–	+	+
C.I. Direct Brown 2	87	–	?	NT	NT
C.I. Direct Brown 95	89	–	?	+	+
C.I. Direct Green 1	ND	–	+w	NT	NT
3,3′-Dimethylbenzidine	99	–	+	+	+
C.I. Acid Red 114	85	–	+	+	+
C.I. Direct Blue 25	ND	–	–	+	+
C.I. Direct Blue 53	69	–	?	NT	NT
C.I. Direct Red 2	>92	–	–	+	+
C.I. Direct Red 39	ND	–	–	+	+
3,3′-Dimethoxybenzidine	99	–	+	+	+
C.I. Direct Blue 1	ND	–	–	+	NT
C.I. Direct Blue 8	30	–	–	+	+
C.I. Direct Blue 10	48	–	–	+	+
C.I. Direct Blue 15	50	–	–	+	+
C.I. Direct Violet 32	83	–	–	+	+
3,3′-Dihydroxybenzidine	ND	–	+	NT	NT
C.I. Direct Blue 218	60	–	–	–	–
3,3′-Dichlorobenzidine	>99	+	+	+	+
C.I. Pigment Yellow 12	ND	–	–	–	–

[a] The data supporting these summaries can be found in Prival and Mitchell (1982), Reid *et al.* (1984a,b), Mortelmans *et al.* (1986), Zeiger *et al.* (1987, 1988), and NTP (unpublished).

[b] None = no metabolic activation conditions used; S9 = Standard (aerobic) preincubation test procedure; FMN (flavin mononucleotide) = FMN-supplemented S9 for reductive metabolism; cecal = rat cecal flora suspension for anaerobic metabolism. – = not mutagenic, + = mutagenic, +w = weakly mutagenic, ? = equivocal response, NT = not tested.

[c] ND = not determined

The principal toxic effects associated with the administration of C.I. Direct Blue 218 in the 13-week studies occurred primarily in the liver of male and female rats and the kidney of female rats receiving 20,000 ppm, and in the liver and spleen of male and female mice receiving 20,000 ppm. Hepatic lesions in rats included pigmented periportal Kupffer cells, minimal to mild individual hepatocyte necrosis, increased numbers of binucleated and multinucleated hepatocytes, and minimal bile duct hyperplasia; renal lesions consisted of increased mineral microconcretions along the corticomedullary junction. Hepatic lesions in mice included centrilobular hepatocyte hypertrophy and karyomegaly, multifocal hepatocyte necrosis, oval cell proliferation, and pigmented macrophages; increased numbers of pigmented macrophages occurred in the spleen. Hematocrit, hemoglobin, mean erythrocyte volume, and mean erythrocyte hemoglobin were significantly lower, and serum alanine aminotransferase and sorbitol dehydrogenase levels were significantly higher in exposed animals, which is indicative of the hepatic injury. Because of the body weight effects and liver toxicity in male and female rats and mice receiving 20,000 ppm, the high dose selected for the 2-year studies was 10,000 ppm. In the 2-year studies, there was again some depression of body weights and toxicity to the liver and hematologic systems, but this toxicity was not severe and there was no statistically significant difference in survival among groups of exposed rats and mice and the respective control groups.

All five of the chemicals/dyes tested in this Initiative (3,3′-dimethoxybenzidine, 3,3′-dimethylbenzidine, C.I. Direct Blue 15, C.I. Acid Red 114, and C.I. Direct Blue 218) caused some degree of liver toxicity, although the metabolite responsible for these toxic effects has not been identified. The spectrum of liver toxicity observed was similar for these chemicals/dyes and included elevated serum enzyme levels, increased liver weights, liver necrosis and/or other toxic lesions. This suggests that this toxicity is due to the aromatic components/metabolites.

The administration of 10,000 or 20,000 ppm C.I. Direct Blue 218 to rats and mice resulted in mild but significant reductions in the hematocrit, hemoglobin, mean erythrocyte volume, and/or mean erythrocyte hemoglobin values. This was an indication of a mild microcytic normochromic anemia. The presence of increased numbers of pigmented macrophages in the liver of rats and mice and the spleen of mice and the presence of pigment in the renal tubule epithelium of rats suggest that the hematologic differences may have been related to hematolysis. C.I. Acid Red 114 also caused decreases in hematocrit, hemoglobin, and erythrocyte counts in male or female rats. The administration of C.I. Acid Red 114 for 2 weeks resulted in the reduction in cellularity of the bone marrow at the highest doses. Direct toxicity may therefore be an alternative pathogenesis for the mild hematologic toxicity caused by administration of some of the benzidine dyes.

Of the dyes tested in this Initiative only C.I. Direct Blue 218 contains copper. While copper at 5 ppm serves as an essential element in hemoglobin formation and normal erythropoiesis, higher doses can cause liver and hematologic toxicity in the rodent (Barka *et al.*, 1964; NAS, 1977; Sternlieb, 1980; ATSDR, 1990). In the NTP 13-week feed studies of copper sulfate, liver and hematologic toxicity was observed in rats primarily at 4,000 and 8,000 ppm, exposure levels that are higher than that delivered with C.I. Direct Blue 218 (10,000 ppm dye = 900 ppm total copper or 57 mg copper/kg body weight per day for the rats). Liver and hematologic toxicity was not observed in mice in the NTP copper sulfate studies (NTP, 1993). In other studies, diets containing 1,000 ppm copper as copper sulfate administered for 21 days to rats inhibited weight gain by approximately 30% and resulted in increased liver weights (Boyden *et al.*, 1938). Intraperitoneal injections of copper sulfate at 3.74 mg copper/kg body weight per day caused an accumulation of copper in liver lysosomes and damage to the lysosomal cell membranes with release of acid hydroxylases and other liver enzymes (Lindquist, 1967, 1968; Lal *et al.*, 1974). The hematologic toxicity observed in rats administered copper sulfate was consistent with a microcytic anemia possibly due to interference with iron metabolism (NTP, 1993). The liver and hematologic toxicity observed with C.I. Direct Blue 218 appears to be due primarily to the aromatic components, but the combination of copper and aromatic components may also be a factor in the liver and hematologic toxicity observed.

In these studies of C.I. Direct Blue 218, the principal neoplastic finding in rats occurred in the pharynx where squamous cell papillomas occurred in five males and two females receiving 10,000 ppm. One squamous cell carcinoma and one basosquamous

tumor were seen in two additional 10,000 ppm male rats. Two male rats that received 10,000 ppm had focal, nonneoplastic proliferations (squamous hyperplasia) of the mucosal epithelium in the posterior pharynx. These findings were considered to represent some evidence for a carcinogenic response in male rats because the incidence of squamous cell papillomas of the pharynx in the 10,000 ppm group was significantly greater than that in the control group and exceeded the incidence in the NTP historical database. The evidence for a carcinogenic response was not considered to be strong enough to place it in the clear evidence category because the incidence of squamous cell carcinomas of the pharynx was not significantly increased. The evidence for a treatment-related neoplastic response in the pharynx of male rats is supported by the fact that the other benzidine dyes also cause neoplasms of the oral cavity (Table 21). In contrast to the findings in male rats, the incidence of squamous cell papillomas of the pharynx was not significantly increased in female rats.

C.I. Direct Blue 218 caused an increased incidence of basal cell hyperplasia in the forestomach of male rats that received 3,000 and 10,000 ppm and in female rats that received 10,000 ppm. Although the difference was not significant, more male rats that received 3,000 and 10,000 ppm had squamous cell hyperplasia than did controls. Squamous papillomas of the forestomach occurred in two male rats receiving 3,000 ppm, one male rat receiving 10,000 ppm, and one squamous cell carcinoma of the forestomach occurred in a male rat receiving 3,000 ppm. No squamous cell neoplasms were observed in the control or 1,000 ppm groups of male rats. Squamous cell neoplasms of the forestomach are relatively uncommon (4/1,253, 0.3%) and the occurrence of three squamous cell neoplasms at 3,000 ppm and one at 10,000 ppm in male rats may have been related to chemical administration. The evidence for squamous cell neoplasms in male rats was not strong enough to place it in the some evidence category because the incidences were low and not significant. There was no evidence for a treatment-related carcinogenic response in female rats, because only one squamous cell neoplasm was observed at 3,000 ppm.

The incidences of uterine endometrial stromal polyps were increased in all exposed groups of female rats. However, the incidences did not increase with exposure level, the incidence in the concurrent control group was abnormally low, and the incidences were within the historical range for this neoplasm. Therefore, these neoplasms were not considered to be clearly related to treatment.

C.I. Direct Blue 218 did not induce the wide spectrum of carcinogenic activity in the rat observed with 3,3′-dimethylbenzidine dihydrochloride, 3,3′-dimethoxybenzidine dihydrochloride, C.I. Direct Blue 15, or C.I. Acid Red 114 (refer to Table 22 for a comparison of doses used). With 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, C.I. Direct Blue 15, and C.I. Acid Red 114, treatment-related neoplasms occurred in the skin, Zymbal's gland, oral cavity epithelium, liver, preputial and clitoral glands, and intestine of both male and female rats. A few of these neoplasms were observed as early as 9 months, and the numbers of neoplasms at the various sites increased with increasing length of exposure. This relatively lower carcinogenic effect of C.I. Direct Blue 218 may be related to the decreased metabolism and absorption of the metallized dye or decreased mutagenic activity of dye components. The benzidine congener and a putative metabolite of the major component in C.I. Direct Blue 218 is 3,3′-dihydroxybenzidine, which was mutagenic in *Salmonella* (NTP unpublished data). Comparison of the mutagenic responses of this congener to the test responses of C.I. Direct Blue 218 shows that the highest dose tested of C.I. Direct Blue 218 was 0.5 μmol/plate, whereas the mutagenic responses of 3,3′-dihydroxybenzidine were seen only at doses above 1.5 μmol/plate.

There was clear evidence of carcinogenicity in the liver of male and female mice, although there was no evidence for a carcinogenic response at this site in rats. In male and female mice receiving 10,000 ppm, there were significantly increased incidences of combined hepatocellular foci, hepatocellular adenoma, and hepatocellular adenoma and/or carcinoma (combined). The strength of this carcinogenic response in the liver of mice was supported by the fact that the number of mice with multiple hepatocellular adenomas was also significantly increased in the 10,000 ppm groups. The incidences of hepatocellular adenomas and carcinomas in the 10,000 ppm groups exceeded the incidences in the concurrent controls and the range for these neoplasms in the historical database. Hepatocellular adenoma or carcinoma (combined) occurred in 36% of control males (Table C4a) and 16% of control females (Table D4a), while in the 10,000 ppm groups

TABLE 21
Incidences of Treatment-Related Neoplasms in the National Toxicology Program's Benzidine Dye Initiative Studies

Males	Females
Neoplasms in F344/N Rats in the 21-Month Drinking Water Studies of 3,3′-Dimethoxybenzidine Dihydrochloride (doses: 0, 80, 170, and 330 ppm)	
Skin basal cell or sebaceous gland neoplasms: 2/60, 33/45, 56/75, 41/60	Skin basal cell neoplasms: 0/60, 4/45, 3/75, 2/60
Skin squamous cell neoplasms: 0/60, 13/45, 28/75, 22/60	Zymbal's gland neoplasms: 1/60, 12/45, 21/75, 16/60
Zymbal's gland neoplasms: 0/59, 10/45, 25/75, 30/60	Clitoral gland neoplasms: 7/58, 27/44, 48/74, 41/55
Preputial gland neoplasms: 16/60, 12/43, 33/73, 29/59	Palate or tongue neoplasms: 2/60, 2/45, 6/75, 5/60
Palate or tongue neoplasms: 1/60, 8/45, 10/75, 11/60	Large intestine neoplasms: 0/60, 1/45, 1/75, 3/60
Small intestine neoplasms: 0/60, 4/45, 7/75, 5/60	Liver neoplasms: 0/60, 1/44, 0/75, 3/60
Large intestine neoplasms: 0/60, 1/45, 8/75, 8/60	Mammary gland adenocarcinomas: 1/60, 2/45, 14/75, 20/60
Liver neoplasms: 1/60, 4/45, 7/74, 8/60	Uterus or cervix neoplasms: 0/60, 4/45, 2/75, 2/60
Mesotheliomas: 2/60, 1/45, 7/75, 6/60	
Neoplasms in F344/N Rats in the 15-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride (doses: 0, 30, 70, and 150 ppm)	
Skin basal cell neoplasms: 0/60, 11/45, 54/75, 30/60	Skin basal cell neoplasms: 0/60, 3/45, 10/75, 9/60
Skin sebaceous cell adenoma: 0/60, 0/45, 7/75, 5/60	Skin squamous cell neoplasms: 0/60, 3/45, 9/75, 12/60
Skin keratoacanthomas: 1/60, 1/45, 8/75, 5/60	Zymbal's gland neoplasms: 0/57, 6/44, 32/73, 42/60
Skin squamous cell neoplasms: 0/60, 2/45, 17/75, 27/60	Clitoral gland neoplasms: 0/60, 14/45, 42/75, 32/59
Zymbal's gland neoplasms: 1/59, 3/45, 32/75, 36/59	Liver neoplasms: 0/60, 0/45, 7/74, 4/60
Preputial gland neoplasms: 2/60, 4/45, 6/75, 9/60	Oral cavity neoplasms: 0/60, 3/45, 9/75, 13/60
Liver neoplasms: 0/60, 0/45, 35/75, 33/60	Small intestine neoplasms: 0/60, 1/45, 3/75, 5/60
Oral cavity neoplasms: 0/60, 0/45, 4/75, 5/60	Large intestine neoplasms: 0/60, 1/45, 7/75, 4/60
Small intestine neoplasms: 0/60, 0/45, 4/75, 8/60	Mammary gland adenocarcinoma: 0/60, 1/45, 3/75, 6/60
Large intestine neoplasms: 0/60, 0/45, 6/75, 15/60	Lung neoplasms: 1/60, 1/45, 3/74, 4/60
Lung neoplasms: 1/60, 0/45, 8/75, 6/60	
Mesothelioma: 0/60, 0/45, 3/75, 4/60	
Neoplasms in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (doses: 0, 630, 1,250, and 2,500 ppm)	
Skin basal cell neoplasms: 2/50, 9/35, 27/65, 28/50	Skin squamous cell neoplasms: 0/50, 2/35, 6/65, 5/50
Skin sebaceous cell adenoma: 0/50, 1/35, 7/65, 3/50	Zymbal's gland neoplasms: 0/50, 4/35, 11/65, 17/50
Skin keratoacanthomas: 2/50, 1/35, 7/65, 2/50	Clitoral gland neoplasms: 7/50, 11/31, 24/64, 27/50
Skin squamous cell neoplasms: 2/50, 4/35, 11/65, 19/50	Liver neoplasms: 0/50, 0/35, 2/65, 5/50
Zymbal's gland neoplasms: 1/50, 5/35, 10/65, 20/50	Oral cavity neoplasms: 2/50, 4/35, 19/65, 15/50
Preputial gland neoplasms: 8/49, 5/35, 23/64, 9/48	Small intestine adenocarcinoma: 0/50, 0/35, 1/65, 3/50
Liver neoplasms: 0/50, 6/35, 9/65, 11/50	Large intestine adenomatous polyp: 0/50, 0/35, 3/65, 1/50
Oral cavity neoplasms: 1/50, 10/35, 24/65, 17/50	Uterine neoplasms: 1/50, 0/35, 1/65, 4/50
Small intestine neoplasms: 0/50, 1/35, 0/65, 2/50	Mononuclear cell leukemia: 7/50, 13/35, 27/65, 15/50
Large intestine neoplasms: 0/50, 1/35, 6/65, 8/50	

(continued)

TABLE 21
Incidences of Treatment-Related Neoplasms in the National Toxicology Program's Benzidine Dye Initiative Studies (continued)

Males	Females
Neoplasms in F344/N Rats in the 24-Month Drinking Water Studies of C.I. Acid Red 114 (doses male: 0, 70, 150, and 300 ppm; doses female: 0, 150, 300, and 600 ppm)	
Skin basal cell neoplasms: 1/50, 5/35, 28/65, 32/50	Skin basal cell neoplasms: 0/50, 4/35, 7/65, 5/50
Skin keratoacanthoma: 1/50, 1/35, 4/65, 7/50	Zymbal's gland neoplasms: 0/50, 3/35, 18/65, 19/50
Skin sebaceous cell neoplasms: 1/50, 1/35, 5/65, 6/50	Clitoral gland neoplasms: 11/48, 17/32, 28/62, 23/50
Skin squamous cell neoplasms: 1/50, 2/35, 11/65, 9/50	Liver neoplasms: 0/50, 0/35, 19/64, 8/50
Zymbal's gland neoplasms: 0/50, 0/35, 8/65, 7/50	Lung neoplasms: 1/50, 2/35, 9/65, 4/50
Liver neoplasms: 2/50, 2/35, 15/65, 20/50	Oral cavity epithelium neoplasms: 0/50, 3/35, 9/65, 6/50
	Small intestine neoplasms: 0/50, 0/35, 1/65, 2/50
	Large intestine neoplasms: 0/50, 1/35, 0/65, 3/50
Neoplasms in F344/N Rats in the 24-Month Feed Studies of C.I. Direct Blue 218 (doses: 0, 1,000, 3,000, and 10,000 ppm)	
Pharynx neoplasms: 0/50, 0/50, 0/50, 7/50	None
Neoplasms in B6C3F. Mice in the 24-Month Feed Studies of C.I. Direct Blue 218 (doses: 0, 1,000, 3,000, and 10,000 ppm)	
Liver neoplasms: 21/50, 20/50, 23/50, 45/50	Liver neoplasms: 10/49, 15/50, 21/49, 45/49

TABLE 22
Estimated Amount of Benzidine Congener or Dye Consumed in Long Term Studies

Benzidine Congener	Males			Females		
	Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
Rats						
3,3′-Dimethylbenzidine Dihydrochloride						
Drinking Water (ppm)[a]	30	70	150	30	70	150
Dose (μmol/kg/day)[b]	5	13	74	6	20	54
3,3′-Dimethoxybenzidine Dihydrochloride						
Drinking Water (ppm)	80	170	330	80	170	330
Dose (μmol/kg/day)	18	38	66	22	44	72
C.I. Acid Red 114						
Drinking Water (ppm)	70	150	300	150	300	600
Dose (μmol/kg/day)	4	9	23	11	25	82
C.I. Direct Blue 15						
Drinking Water (ppm)	630	1,250	2,500	630	1,250	2,500
Dose (μmol/kg/day)	62	89	215	49	100	198
C.I. Direct Blue 218						
Feed (ppm)	1,000	3,000	10,000	1,000	3,000	10,000
Dose (μmol/kg/day)	33	100	355	37	109	374
Mice						
C.I. Direct Blue 218						
Feed (ppm)	1,000	3,000	10,000	1,000	3,000	10,000
Dose (μmol/kg/day)	96	295	1,292	117	358	1,810

[a] Dose in drinking water or feed
[b] Based on water or feed consumption measurements during the last year of the study, and molecular weight for congeners or major component of dye. The molecular weight for each congener or major component is: 3,3′-dimethylbenzidine dihydrochloride, 285; 3,3′-dimethoxybenzidine dihydrochloride, 317; C.I. Acid Red 114, 830; C.I. Direct Blue 15, 993; C.I. Direct Blue 218, 1,090.

90% to 92% of the mice had these neoplasms. This was considered to be clear evidence for a carcinogenic response because the incidences of both hepatocellular adenoma and carcinoma were increased in both males and females.

Carcinomas of the small intestine were observed in one male mouse receiving 10,000 ppm at the 15-month interim evaluation and in three males receiving 10,000 ppm at the end of the 2-year study. A carcinoma of the small intestine was also seen in one control male and an adenoma in one male that received 10,000 ppm. Spontaneous neoplasms of the small intestine are rare in mice. The occurrence of neoplasms of the small intestine in these studies may have been biologically significant because the incidence in male mice at 10,000 ppm exceeded the level for this neoplasm in the historical database (12/1,374, 0.9%; range 0%-4%). The evidence was not considered to be strong enough to place it into the some evidence category because the incidences were low and not statistically significant. One carcinoma of the small intestine was seen in a female mouse receiving 3,000 ppm and another in a female receiving 10,000 ppm. Because the incidence of this neoplasm was within the historical range (0%-6%; 8/1,371, 0.6%), this was not considered to be biologically significant.

A few kidney neoplasms were observed in exposed male mice: two renal tubule adenomas and one renal tubule carcinoma at 1,000 ppm, one renal tubule adenoma at 3,000 ppm, and one renal tubule adenoma at 10,000 ppm. Renal tubule neoplasms are rare in the historical control male mice (4/1,366, 0.3%), and the occurrence of three renal tubule neoplasms at 1,000 ppm exceeded the historical incidence for this neoplasm and may have been related to chemical administration. The evidence was not strong enough to be considered some evidence of a carcinogenic response because the number of neoplasms was low and the incidence was not statistically significant or dose related.

C.I. Direct Blue 218 and benzidine both produce hepatocellular neoplasms in mice although benzidine has been reported to produce this effect at much lower doses than the 10,000 ppm level used in the C.I. Direct Blue 218 studies. Littlefield *et al.* (1983) found hepatic neoplasms when benzidine was administered to F_1 hybrid and monohybrid mice from a BALB/c male and C57BL/6 female cross at doses ranging from 20 to 160 ppm in the drinking water for the lifetime of the animals. Vesselinovitch *et al.* (1975) administered benzidine at levels of 50, 100, and 150 ppm in the feed to $B6C3F_1$ mice for 84 weeks, and hepatic, pulmonary, and harderian gland neoplasms were observed at all dose levels. Benzidine administered in the drinking water at doses of 30 to 40 ppm to hybrid mice (BALB/c × C57BL/6) resulted in hepatic neoplasms by 18 months at all dose levels, and neoplasms were detected as early as 9 months (Nelson *et al.*, 1982). While the hepatic neoplasms produced in mice by C.I. Direct Blue 218 and benzidine are similar, the differences in the doses required to produce these neoplasms suggest that only a small percentage of the total dose of C.I. Direct Blue 218 results in the formation of an active metabolite.

The C.I. Direct Blue 218 component responsible for producing hepatic neoplasms may be structurally related to the benzidine metabolite associated with the induction of hepatic neoplasms. Results from rodent studies indicate that N-acetylation is the first step in the metabolism for benzidine-induced initiation of liver carcinogenesis (Morton *et al.*, 1981; Martin *et al.*, 1982; Kennelly *et al.*, 1984; Frederick *et al.*, 1985). Administration of benzidine or acetylbenzidine to rats or mice produced a single hepatic metabolite-DNA adduct, which upon enzymatic hydrolysis was identified as N-(deoxyguanosin-8-yl)-N′-acetylbenzidine (Kennelly *et al.*, 1984). Formation of benzidine-DNA adducts has been associated with *in vitro* genotoxicity. Talaska *et al.* (1987) and Phillips *et al.* (1990) have also shown that the level of benzidine-DNA adduct in the mouse liver correlated with the number of chromosomal aberrations in the hepatocytes. Metabolites of C.I. Direct Blue 218 may also form DNA adducts in the mouse liver leading to genetic alterations that would allow the cells to escape normal growth control mechanisms and result in a neoplastic response.

While treatment of $B6C3F_1$ mice with either C.I. Direct Blue 218 or benzidine results in hepatocellular neoplasms, liver neoplasms were not seen in BALB/c mice treated with benzidine-based compounds. Studies conducted by Schieferstein *et al.* (1989) using BALB/c mice treated with 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine did not result in hepatic neoplasms. The BALB/c mouse has been shown to be less susceptible than the $B6C3F_1$ mouse to at least one other hepatic carcinogen, urethane

(Dragani *et al.*, 1991). Genetic differences between BALB/c mice and $B6C3F_1$ mice may contribute to differences in susceptibility to the induction of mouse hepatic neoplasms by the benzidine chemicals/dyes. Buchmann *et al.* (1991) suggested that mutational activation of the *ras* gene and differences in the susceptibility to activation may contribute to differences in the susceptibility of various rodent strains to chemical-induced hepatic neoplasms.

The process of carcinogenesis may involve a number of distinct steps, such as the activation of proto-oncogenes and the loss of specific regulatory substances such as suppressor genes (Barrett *et al.*, 1987). Some support for this model comes from rodent studies of hepatic neoplasms. Chemical induction of hepatic neoplasms in mice has been associated with activating point mutations in *ras* genes (Reynolds *et al.*, 1990; Buchmann *et al.*, 1991; Goodman *et al.*, 1991). One factor that may affect the susceptibility of a mouse to chemical induction of hepatic neoplasms is the background level of hypomethylation in the H-*ras* oncogene, because the level of methylation of a gene has been associated with control of gene expression (Holliday, 1987; Jones and Buckley, 1990). Hepatic DNA from $B6C3F_1$ mice and the parent strain C3H/HeN is hypomethylated and both strains have a high background level of hepatic neoplasms (Goodman *et al.*, 1991). In contrast, hypomethylation was not associated with the hepatic DNA from C57BL/6 mice, a strain with a low incidence of hepatic neoplasms (Goodman *et al.*, 1991). The hypomethylation associated with hepatic DNA in mouse strains such as $B6C3F_1$ may be close to the threshold of hypomethylation required to activate the proto-oncogene, and exposure to certain chemicals may then cross the threshold, thus activating the oncogene and resulting in the development of a neoplasm. This model may explain why these mice are more susceptible to benzidine-induced hepatic neoplasms (Vorce and Goodman, 1989; Goodman *et al.*, 1991).

Benzidine exposure in humans is associated with urinary bladder neoplasms while in rodents C.I. Direct Blue 218 or benzidine/dye exposure is associated with hepatic neoplasms or neoplasms at other sites. Species such as rodents that rapidly acetylate aromatic amines develop hepatic neoplasms, while species which are relatively slow acetylators develop urinary bladder neoplasms (Lower and Bryan, 1973). The formation of urinary bladder neoplasms in dogs and humans probably occurs via a metabolic pathway that differs from that which leads to hepatic neoplasms in rodents (Wise *et al.*, 1984; Lakshmi *et al.*, 1990). Yamazoe *et al.* (1988) studied the binding of benzidine to calf thymus DNA and showed that a series of peroxidases can oxidize benzidine by a common peroxidative mechanism to form benzidine diimine, an electrophilic metabolite capable of binding to DNA. A major adduct derived was N3-(deoxyguanosin-N7,C8-yl)-benzidine, which may be the DNA adduct formed in the urinary bladder of humans and dogs. The authors indicated that additional studies would be needed to quantify the degree of DNA cross linking and adduct formation *in vitro* and *in vivo* that is associated with benzidine carcinogenesis (and carcinogenesis from related chemicals/dyes such as C.I. Direct Blue 218) in individual tissues.

Studies by Ashby and Richardson (1985) have shown that exposure to established human carcinogens, including benzidine, was associated with a clastogenic response in human peripheral lymphocytes. Perera *et al.* (1988) found that polycyclic aromatic hydrocarbon-DNA adducts were increased in foundry workers exposed to these chemicals. Mirkova and Lalchev (1990) found that there was an increase in the frequency of chromosomal aberrations in peripheral lymphocytes of workers exposed to benzidine and benzidine dyes in a plant manufacturing Direct Black 38 and Direct Blue 6. The findings from both human and animal studies suggest that there is an association between the level of benzidine-DNA adducts and the carcinogenic action of the benzidine dye.

Exposure to aromatic amines (benzidine, 4-aminobiphenyl, naphthylamine, and *o*-toluidine) has been associated with cancer in humans (Lutz, 1984; Tomatis *et al.*, 1989; Ward *et al.*, 1991). An aromatic amine is considered to be a "structural alert" for chemical carcinogenicity (Ashby and Tennant, 1991). The benzidine-based dyes including C.I. Direct Blue 218, C.I. Direct Blue 15, and C.I. Acid Red 114 contain chemicals that can be metabolized to aromatic amines. These chemicals have also been found to be carcinogenic in rodents. These findings are consistent with the association of exposure to aromatic amines and the development of cancer in humans.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity** of C.I. Direct Blue 218 in male F344/N rats based on the occurrence of pharyngeal neoplasms. Squamous cell neoplasms of the forestomach may have been chemical related. There was *no evidence of carcinogenic activity* of C.I. Direct Blue 218 in female F344/N rats given 1,000, 3,000, or 10,000 ppm. There was *clear evidence of carcinogenic activity* of C.I. Direct Blue 218 in male and female $B6C3F_1$ mice based on increased incidences of hepatocellular adenomas and carcinomas. The occurrence of a few neoplasms of the small intestine and kidney in male mice may have been related to C.I. Direct Blue 218 treatment.

The administration of C.I. Direct Blue 218 produced an increased incidence of forestomach basal cell hyperplasia in rats and hepatocellular foci of cytologic alteration in mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF C.I. DIRECT BLUE 218

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	9
Early deaths				
Moribund	13	22	16	18
Natural deaths	7	3	5	9
Survivors				
Terminal sacrifice	30	25	29	24
Animals examined microscopically	60	60	60	59
15-Month Interim Evaluation				
Alimentary System				
None				
Cardiovascular System				
None				
Endocrine System				
Adrenal gland, medulla	(10)	(10)	(10)	(9)
Pheochromocytoma				1 (11%)
Pituitary gland	(9)	(10)	(10)	(9)
Pars distalis, adenoma			1 (10%)	2 (22%)
General Body System				
None				
Genital System				
Testes	(10)	(10)	(10)	(9)
Interstitial cell, adenoma	4 (40%)	4 (40%)		4 (44%)
Bilateral, interstitial cell, adenoma	5 (50%)	3 (30%)	3 (30%)	
Hematopoietic System				
Spleen	(10)	(10)	(10)	(9)
Leukemia mononuclear	1 (10%)			
Integumentary System				
None				
Musculoskeletal System				
None				

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(9)
Alveolar/bronchiolar adenoma				1 (11%)
Special Senses System				
None				
Urinary System				
None				
Systemic Lesions				
None				
2-Year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Leiomyoma	1 (2%)			
Sarcoma, metastatic, spleen			1 (2%)	
Intestine large, rectum	(46)	(49)	(49)	(50)
Intestine large, cecum	(46)	(49)	(49)	(49)
Adenocarcinoma				1 (2%)
Intestine small, duodenum	(49)	(49)	(50)	(48)
Sarcoma, metastatic, spleen			1 (2%)	
Intestine small, jejunum	(47)	(49)	(49)	(46)
Intestine small, ileum	(46)	(49)	(48)	(47)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	4 (8%)	5 (10%)	1 (2%)	6 (12%)
Hepatocellular adenoma, multiple	1 (2%)	1 (2%)	1 (2%)	
Histiocytic sarcoma				2 (4%)
Sarcoma, metastatic, spleen			1 (2%)	
Mesentery	(5)	(5)	(7)	(4)
Sarcoma, metastatic, spleen			1 (14%)	
Pancreas	(50)	(50)	(50)	(50)
Sarcoma, metastatic, spleen			1 (2%)	
Pharynx		(1)		(9)
Palate, basosquamous tumor benign				1 (11%)
Palate, squamous cell carcinoma				1 (11%)
Palate, squamous cell papilloma				5 (56%)
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Sarcoma, metastatic, spleen			1 (2%)	
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma			2 (4%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)		1 (2%)	
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma				1 (2%)
Sarcoma, metastatic, spleen			1 (2%)	
Adrenal medulla	(49)	(50)	(50)	(50)
Mixed tumor malignant			1 (2%)	
Pheochromocytoma malignant	2 (4%)		2 (4%)	1 (2%)
Pheochromocytoma benign	14 (29%)	7 (14%)	9 (18%)	7 (14%)
Sarcoma, metastatic, spleen			1 (2%)	
Bilateral, pheochromocytoma benign	1 (2%)	2 (4%)	3 (6%)	5 (10%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	5 (10%)	3 (6%)	7 (14%)
Carcinoma			1 (2%)	
Parathyroid gland	(43)	(47)	(46)	(45)
Adenoma		1 (2%)	1 (2%)	
Pituitary gland	(50)	(50)	(48)	(50)
Pars distalis, adenoma	15 (30%)	14 (28%)	15 (31%)	9 (18%)
Pars distalis, adenoma, multiple			1 (2%)	3 (6%)
Pars distalis, carcinoma	2 (4%)			
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	7 (14%)	1 (2%)	4 (8%)	4 (8%)
C-cell, carcinoma	1 (2%)		1 (2%)	1 (2%)
Follicular cell, carcinoma			1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Squamous cell papilloma				1 (2%)
Bilateral, adenoma	1 (2%)			
Prostate	(50)	(50)	(50)	(49)
Seminal vesicle	(50)	(50)	(50)	(49)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Genital System (continued)				
Testes	(50)	(50)	(50)	(50)
Sarcoma, metastatic, spleen			1 (2%)	
Bilateral, interstitial cell, adenoma	41 (82%)	41 (82%)	43 (86%)	33 (66%)
Interstitial cell, adenoma	4 (8%)	5 (10%)	5 (10%)	11 (22%)
Hematopoietic System				
Blood		(1)		
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma				2 (4%)
Lymph node	(15)	(16)	(23)	(18)
Pancreatic, rhabdomyosarcoma, metastatic, skeletal muscle	1 (7%)			
Lymph node, mandibular	(48)	(50)	(49)	(50)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)			
Sarcoma, metastatic, spleen			1 (2%)	
Spleen	(50)	(50)	(50)	(49)
Sarcoma			1 (2%)	1 (2%)
Thymus	(48)	(40)	(49)	(48)
Integumentary System				
Mammary gland	(48)	(49)	(50)	(46)
Adenocarcinoma				1 (2%)
Fibroadenoma	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Fibroadenoma, multiple		1 (2%)		
Histiocytic sarcoma				1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	2 (4%)	2 (4%)		
Basal cell carcinoma		1 (2%)		
Basosquamous tumor benign		1 (2%)		
Keratoacanthoma	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Keratoacanthoma, multiple		1 (2%)		
Squamous cell papilloma	1 (2%)			1 (2%)
Sebaceous gland, adenoma			1 (2%)	1 (2%)
Subcutaneous tissue, fibroma	4 (8%)	4 (8%)	5 (10%)	2 (4%)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, histiocytic sarcoma				1 (2%)
Subcutaneous tissue, neurofibrosarcoma	1 (2%)			
Musculoskeletal System				
Skeletal muscle	(2)	(1)		(1)
Histiocytic sarcoma				1 (100%)
Lipoma		1 (100%)		
Abdominal, rhabdomyosarcoma	1 (50%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Ependymoma benign		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)		1 (2%)	2 (4%)
Histiocytic sarcoma				1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla			1 (2%)	
Sarcoma, metastatic, spleen			1 (2%)	
Nose	(50)	(50)	(49)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Zymbal's gland	(48)	(48)	(48)	(48)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Squamous cell papilloma	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma			2 (4%)	
Transitional epithelium, carcinoma	1 (2%)	1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				2 (4%)
Leukemia mononuclear	18 (36%)	25 (50%)	23 (46%)	26 (52%)
Mesothelioma malignant	1 (2%)	1 (2%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	49	49
Total primary neoplasms	136	129	137	143
Total animals with benign neoplasms	47	50	49	46
Total benign neoplasms	107	100	102	106
Total animals with malignant neoplasms	27	27	28	31
Total malignant neoplasms	29	29	35	37
Total animals with metastatic neoplasms	1		2	
Total metastatic neoplasms	2		12	

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm

Number of Days on Study	2 7 3	4 3 8	5 0 6	5 3 9	5 4 8	5 7 0	5 7 2	5 7 2	5 8 1	6 0 7	6 1 4	6 1 8	6 2 9	6 3 0	6 7 3	6 7 3	6 7 9	6 8 2	6 9 3	7 1 5	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 1 1	0 2 1	0 5 3	0 4 2	0 5 1	0 7 1	0 6 2	0 9 5	0 4 1	0 9 2	0 7 2	1 0 1	0 4 3	0 3 4	0 5 4	0 8 2	0 7 4	1 0 2	0 3 1	0 6 5	0 1 2	0 1 3	0 1 4	0 1 5	0 2 2
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																								
Intestine large, rectum	A	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																						X		X
Hepatocellular adenoma, multiple																								
Mesentery											+													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant						X																		
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																		X						
Pheochromocytoma benign														X	X	X				X		X	X		X
Bilateral, pheochromocytoma benign																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X												
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X						X	X			X	X			X		X
Pars distalis, carcinoma			X	X																				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma					X				X							X				X	X			
C-cell, carcinoma																								
General Body System																								
None																								
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Bilateral, adenoma																								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma				X	X	X	X		X	X	X	X	X	X		X	X	X		X	X	X		X	X
Interstitial cell, adenoma																			X				X		

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 2	0 3 3	0 3 5	0 4 4	0 4 5	0 5 2	0 5 5	0 6 1	0 6 3	0 6 4	0 7 3	0 7 5	0 8 1	0 8 3	0 8 4	0 8 5	0 9 1	0 9 3	0 9 4	1 0 3	1 0 4	1 0 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma			X																							1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma							X													X						4
Hepatocellular adenoma, multiple																X										1
Mesentery		+										+					+					+				5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																X										2
Pheochromocytoma benign		X	X	X									X					X			X		X			14
Bilateral, pheochromocytoma benign															X											1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X			X							X												4
Parathyroid gland	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X			X				X		X			X	X						X				X	15
Pars distalis, carcinoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma											X														X	7
C-cell, carcinoma															X											1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X																					1
Bilateral, adenoma			X																							1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	41
Interstitial cell, adenoma		X				X																				4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	2 7 3	4 3 8	5 0 6	5 3 9	5 4 8	5 7 0	5 7 2	5 7 2	5 8 1	6 0 7	6 1 4	6 1 8	6 2 9	6 3 0	6 7 3	6 7 3	6 7 9	6 8 2	6 9 3	7 1 5	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 1 1	0 2 1	0 5 3	0 4 2	0 5 1	0 7 1	0 6 2	0 9 5	0 4 1	0 9 2	0 7 2	1 0 1	0 4 3	0 3 4	0 5 4	0 8 2	0 7 4	1 0 2	0 3 1	0 6 5	0 1 2	0 1 3	0 1 4	0 1 5	0 2 2
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+		+				+				+	+	+	+	+			+			+	
Pancreatic, rhabdomyosarcoma, metastatic, skeletal muscle									X															
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhabdomyosarcoma, metastatic, skeletal muscle									X															
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																			X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																						X		
Keratoacanthoma																								
Squamous cell papilloma																								
Subcutaneous tissue, fibroma												X												
Subcutaneous tissue, neurofibrosarcoma							X																	
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle									+															
Abdominal, rhabdomyosarcoma									X															
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye																			+		+		+	+
Zymbal's gland	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma													X											
Squamous cell papilloma																X								
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma		X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X				X	X			X			X			X			X	X
Mesothelioma malignant																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 2 3	0 2 4	0 2 5	0 3 2	0 3 3	0 3 5	0 4 4	0 4 5	0 5 2	0 5 5	0 6 1	0 6 3	0 6 4	0 7 3	0 7 5	0 8 1	0 8 3	0 8 4	0 8 5	0 9 1	0 9 3	0 9 4	1 0 3	1 0 4	1 0 5	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+	+	+	+	+																				15
Pancreatic, rhabdomyosarcoma, metastatic, skeletal muscle																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	49
Rhabdomyosarcoma, metastatic, skeletal muscle																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma									X																	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																	X									2
Keratoacanthoma						X																				1
Squamous cell papilloma											X															1
Subcutaneous tissue, fibroma	X	X			X																					4
Subcutaneous tissue, neurofibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+																								2
Abdominal, rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X																	2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																					+					5
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Squamous cell papilloma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X			X			X						X	X		X		X			X	X				18
Mesothelioma malignant		X																								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm

Number of Days on Study	3 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 6 0 1 1 1 2 3 3 3 4 4 5 5 6 7 7 7 7 8 1 1 1 1 2 2 9 1 4 7 8 1 1 7 7 1 6 1 1 0 0 1 1 3 5 3 3 4 5 1 6
Carcass ID Number	1 1 1 1 2 1 1 1 1 1 2 1 1 2 2 2 2 1 1 1 1 1 1 1 1 9 3 8 8 2 3 9 3 4 4 2 5 7 1 1 2 6 6 5 9 8 8 4 8 2 2 5 2 1 1 3 3 4 1 3 5 1 5 4 3 5 5 1 3 1 3 1 5 4
Alimentary System	
Esophagus	+ +
Intestine large, colon	+ +
Intestine large, rectum	+ + + M +
Intestine large, cecum	+ A + + +
Intestine small, duodenum	+ A + + +
Intestine small, jejunum	+ A + + +
Intestine small, ileum	+ A + + +
Liver	+ +
Hepatocellular adenoma	X X X
Hepatocellular adenoma, multiple	
Mesentery	+ +
Pancreas	+ +
Pharynx	
Salivary glands	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ +
Tooth	
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal cortex	+ +
Adrenal medulla	+ +
Pheochromocytoma benign	X X X X
Bilateral, pheochromocytoma benign	X X
Islets, pancreatic	+ +
Adenoma	X X X
Parathyroid gland	+ + + + M + + + + + + + + + + + + + + M + + + +
Adenoma	
Pituitary gland	+ +
Pars distalis, adenoma	X X X X X X X X X
Thyroid gland	+ +
C-cell, adenoma	
General Body System	
None	
Genital System	
Coagulating gland	+
Epididymis	+ +
Preputial gland	+ +
Adenoma	X X X
Prostate	+ +
Seminal vesicle	+ +
Testes	+ +
Bilateral, interstitial cell, adenoma	X X X X X X X X X X X X X X X X X X X X X
Interstitial cell, adenoma	X X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	1 3 4	1 3 5	1 4 2	1 4 3	1 5 1	1 5 2	1 5 4	1 6 2	1 6 3	1 6 4	1 7 2	1 7 3	1 7 4	1 7 5	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 1 1	2 1 2	2 2 2	2 2 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma				X																X						5
Hepatocellular adenoma, multiple																X										1
Mesentery							+	+												+						5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pharynx	+																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+																									1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign		X							X					X				X								7
Bilateral, pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X																			X	5
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Adenoma																									X	1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X								X				X	X	X									14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X																							1
General Body System																										
None																										
Genital System																										
Coagulating gland																										1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X	X	X	X		X	X		X	X		X	X	X	X	X	X	X	X	41
Interstitial cell, adenoma			X								X			X												5

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	3 6 9	6 0 1	6 1 4	6 1 7	6 1 8	6 2 1	6 3 1	6 3 7	6 3 7	6 4 1	6 4 6	6 5 1	6 5 1	6 6 0	6 7 0	6 7 1	6 7 1	6 7 3	6 8 5	7 1 3	7 1 3	7 1 4	7 1 5	7 2 1	7 2 6
Carcass ID Number	1 9 2	1 3 2	1 8 5	1 8 2	2 2 1	1 3 1	1 9 3	1 3 3	1 4 4	1 4 1	2 2 3	1 5 5	1 7 1	2 1 5	2 1 4	2 1 3	2 2 5	1 6 5	1 6 1	1 5 3	1 9 1	1 8 3	1 8 1	1 4 5	1 8 4
Hematopoietic System																									
Blood															+										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+	+					+		+		+		+	+	+	+	+	+	+	+		+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma												X													
Basal cell carcinoma						X																			
Basosquamous tumor benign																									
Keratoacanthoma																					X				
Keratoacanthoma, multiple																									
Subcutaneous tissue, fibroma																								X	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Lipoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ependymoma benign												X													
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye		+																							
Harderian gland		+																							
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Carcinoma		X																							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X			X		X	X	X		X	X	X	X	X	X	X	X				X	X
Mesothelioma malignant																		X							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	1 3 4	1 3 5	1 4 2	1 4 3	1 5 1	1 5 2	1 5 4	1 6 2	1 6 3	1 6 4	1 7 2	1 7 3	1 7 4	1 7 5	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 1 1	2 1 2	2 2 2	2 2 4	Total Tissues/ Tumors
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+																								16
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	M	+	+	+	+	+	M	+	+	M	M	+	+	+	+	M	M	M	M	M	+	+	+	+	+	40
Integumentary System																										
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma		X															X									2
Fibroadenoma, multiple			X																							1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma								X																		2
Basal cell carcinoma																										1
Basosquamous tumor benign																X										1
Keratoacanthoma										X																2
Keratoacanthoma, multiple															X											1
Subcutaneous tissue, fibroma				X	X	X																				4
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle										+																1
Lipoma										X																1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ependymoma benign																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+														+								+			4
Harderian gland																										1
Zymbal's gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X		X	X			X				X				X	X						X		X	25
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm

Number of Days on Study	3 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 5 3 3 8 2 3 4 7 7 7 7 7 8 8 9 9 9 0 0 1 2 2 2 2 2 6 3 7 1 1 2 9 0 1 4 7 7 0 6 2 2 4 0 9 4 5 9 9 9 9
Carcass ID Number	3 2 2 2 3 2 3 3 2 3 2 3 2 2 2 3 3 3 3 3 3 2 2 2 2 3 9 6 5 0 7 4 0 5 3 6 4 8 5 8 1 4 0 2 2 3 5 5 6 6 3 2 3 4 3 5 4 1 5 2 2 5 5 2 1 1 3 2 3 2 4 1 3 1 4
Alimentary System	
Esophagus	+ +
Intestine large, colon	+ +
Sarcoma, metastatic, spleen	X
Intestine large, rectum	+ + A +
Intestine large, cecum	+ + A +
Intestine small, duodenum	+ +
Sarcoma, metastatic, spleen	X
Intestine small, jejunum	+ + + + + A + + + + + + + + + + + + + + + + + + +
Intestine small, ileum	+ + A + + + + + + + A + + + + + + + + + + + + + +
Liver	+ +
Hepatocellular adenoma	
Hepatocellular adenoma, multiple	
Sarcoma, metastatic, spleen	X
Mesentery	+ + + +
Sarcoma, metastatic, spleen	X
Pancreas	+ +
Sarcoma, metastatic, spleen	X
Salivary glands	+ +
Carcinoma	X
Stomach, forestomach	+ +
Sarcoma, metastatic, spleen	X
Squamous cell carcinoma	
Squamous cell papilloma	X
Stomach, glandular	+ +
Tooth	+
Cardiovascular System	
Heart	+ +
Schwannoma malignant	X
Endocrine System	
Adrenal cortex	+ +
Sarcoma, metastatic, spleen	X
Adrenal medulla	+ +
Mixed tumor malignant	X
Pheochromocytoma malignant	
Pheochromocytoma benign	X X X
Sarcoma, metastatic, spleen	X
Bilateral, pheochromocytoma benign	X
Islets, pancreatic	+ +
Adenoma	
Carcinoma	X
Parathyroid gland	+ + + + + + + + M + + + + + + + + M + + + + + + +
Adenoma	
Pituitary gland	+ + + + + + + + + + + + + + + M + + + + + + + + +
Pars distalis, adenoma	X X X X X X
Pars distalis, adenoma, multiple	X
Thyroid gland	+ +
C-cell, adenoma	X X
C-cell, carcinoma	X
Follicular cell, carcinoma	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	26505	26701	26712	26723	26734	26822	26833	26844	26891	26913	26934	26955	27004	27015	27032	27051	27063	27074	27121	27142	27155	27171	27185	27201	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																									1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																									1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma	X																								1
Hepatocellular adenoma, multiple																			X						1
Sarcoma, metastatic, spleen																									1
Mesentery									+						+							+			7
Sarcoma, metastatic, spleen																									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																									1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																									1
Squamous cell carcinoma														X											1
Squamous cell papilloma			X																						2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																									1
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																									1
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																									1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mixed tumor malignant																									1
Pheochromocytoma malignant						X								X											2
Pheochromocytoma benign		X		X										X				X			X	X			9
Sarcoma, metastatic, spleen																									1
Bilateral, pheochromocytoma benign																				X				X	3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X			X	X											3
Carcinoma																									1
Parathyroid gland	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma						X																			1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Pars distalis, adenoma	X			X	X	X	X	X		X				X			X	X							15
Pars distalis, adenoma, multiple																									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma															X			X							4
C-cell, carcinoma																									1
Follicular cell, carcinoma																		X							1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	3 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 5 3 3 8 2 3 4 7 7 7 7 7 8 8 9 9 9 0 0 1 2 2 2 2 2 6 3 7 1 1 2 9 0 1 4 7 7 0 6 2 2 4 0 9 4 5 9 9 9 9
Carcass ID Number	3 2 2 2 3 2 3 3 2 3 2 3 2 2 2 3 3 3 3 3 3 2 2 2 2 3 9 6 5 0 7 4 0 5 3 6 4 8 5 8 1 4 0 2 2 3 5 5 6 6 3 2 3 4 3 5 4 1 5 2 2 5 5 2 1 1 3 2 3 2 4 1 3 1 4
General Body System	
None	
Genital System	
Epididymis	+ +
Preputial gland	+ +
Adenoma	X X
Prostate	+ +
Seminal vesicle	+ +
Testes	+ +
Sarcoma, metastatic, spleen	X
Bilateral, interstitial cell, adenoma	X X X X X X X X X X X X X X X X X X X X X X X X X
Interstitial cell, adenoma	X X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ +
Lymph node, mandibular	+ + + + + + + + + M + + + + + + + + + + + + + + +
Lymph node, mesenteric	+ +
Sarcoma, metastatic, spleen	X
Spleen	+ +
Sarcoma	X
Thymus	+ M + + + +
Integumentary System	
Mammary gland	+ +
Fibroadenoma	X
Skin	+ +
Keratoacanthoma	X
Sebaceous gland, adenoma	X
Subcutaneous tissue, fibroma	X X
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Spinal cord	+
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	
Pheochromocytoma malignant, metastatic, adrenal medulla	
Sarcoma, metastatic, spleen	X
Nose	+ + + + + + + + + + + + + + + + M + + + + + + + +
Trachea	+ +
Special Senses System	
Eye	+ +
Zymbal's gland	+ + + + + + + + + + + + + + M M + + + + + + + + +
Carcinoma	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 6 5	2 7 1	2 7 2	2 7 3	2 7 4	2 8 2	2 8 3	2 8 4	2 9 1	2 9 3	2 9 4	2 9 5	3 0 4	3 0 5	3 1 2	3 1 3	3 1 4	3 1 5	3 2 1	3 2 4	3 2 5	3 3 1	3 3 5	3 4 1	3 4 2	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																										1
Bilateral, interstitial cell, adenoma	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	43
Interstitial cell, adenoma						X		X																X		5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+	+	+					+							+			+		+	+	+		23
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, spleen																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																	X									2
Sebaceous gland, adenoma																										1
Subcutaneous tissue, fibroma							X			X											X					5
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X																					1
Pheochromocytoma malignant, metastatic, adrenal medulla															X											1
Sarcoma, metastatic, spleen																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye			+																							3
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	3 5 6	5 3 3	5 3 7	5 8 1	6 2 1	6 3 2	6 4 9	6 7 0	6 7 1	6 7 4	6 7 7	6 7 7	6 8 0	6 8 6	6 9 2	6 9 2	6 9 4	7 0 0	7 0 9	7 1 4	7 2 5	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 3 3	2 9 2	2 6 3	2 5 4	3 0 3	2 7 5	3 4 4	3 0 1	2 5 5	3 3 2	2 6 2	3 4 5	2 8 5	2 5 2	2 8 1	3 1 1	3 4 3	3 0 2	3 2 3	3 2 2	3 3 4	2 5 1	2 5 3	2 6 1	2 6 4
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X	X	X	X	X							X	X		X		X		X		X	
Mesothelioma malignant						X																		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 6 5	2 7 1	2 7 2	2 7 3	2 7 4	2 8 2	2 8 3	2 8 4	2 9 1	2 9 3	2 9 4	2 9 5	3 0 4	3 0 5	3 1 2	3 1 3	3 1 4	3 1 5	3 2 1	3 2 4	3 2 5	3 3 1	3 3 5	4 1	4 2	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma												X											X			2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X		X		X				X		X							X	X			X	X	X	23
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm

Number of Days on Study	3 0 5	4 5 0	4 6 0	5 0 6	5 0 6	5 3 1	5 3 2	5 6 6	5 8 0	5 8 3	6 0 1	6 1 0	6 1 4	6 4 6	6 4 6	6 6 8	6 8 0	6 8 0	6 8 4	6 9 2	6 9 9	7 0 2	7 0 2	7 0 6	7 2 2
Carcass ID Number	3 8 1	4 6 1	3 9 2	3 7 3	4 6 3	4 4 3	4 2 4	3 7 5	4 4 4	4 0 5	4 2 5	4 3 2	4 4 2	3 8 4	4 0 1	4 5 3	3 7 1	4 0 3	4 0 4	4 5 4	3 9 1	4 1 3	4 2 2	4 4 5	3 9 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																							X		
Intestine small, duodenum	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma											X								X						
Histiocytic sarcoma																				X					
Mesentery							+							+							+	+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																			+				+		
Palate, basosquamous tumor benign																							X		
Palate, squamous cell carcinoma																									
Palate, squamous cell papilloma																			X						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant															X										
Pheochromocytoma benign													X	X											
Bilateral, pheochromocytoma benign																	X		X						
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X																	X		
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma											X	X						X							
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																					X				
C-cell, carcinoma																									
Follicular cell, carcinoma																					X				
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	4 6 2	3 7 2	3 7 4	3 8 2	3 8 3	3 8 5	3 9 3	3 9 5	4 0 2	4 1 1	4 1 2	4 1 4	4 1 5	4 2 1	4 2 3	4 3 1	4 3 3	4 3 4	4 3 5	4 4 1	4 5 1	4 5 2	4 5 5	4 6 4	4 6 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma										X		X					X						X			6
Histiocytic sarcoma																			X							2
Mesentery																										4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pharynx		+				+				+	+				+	+					+					9
Palate, basosquamous tumor benign																										1
Palate, squamous cell carcinoma																					X					1
Palate, squamous cell papilloma						X				X	X				X											5
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma						X																				1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X																				1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign		X				X			X						X									X		7
Bilateral, pheochromocytoma benign										X			X										X			5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X		X					X										X	X			7
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X					X				X		X				X						X				9
Pars distalis, adenoma, multiple													X		X			X								3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma								X	X						X											4
C-cell, carcinoma																						X				1
Follicular cell, carcinoma																										1
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	3 0 5	4 5 0	4 6 0	5 0 6	5 0 6	5 3 1	5 3 2	5 6 6	5 8 0	5 8 3	6 0 1	6 1 0	6 1 4	6 4 6	6 4 6	6 6 8	6 8 0	6 8 0	6 8 4	6 9 2	6 9 9	7 0 2	7 0 2	7 0 6	7 2 2
Carcass ID Number	3 8 1	4 6 1	3 9 2	3 7 3	4 6 3	4 4 3	4 2 4	3 7 5	4 4 4	4 0 5	4 2 5	4 3 2	4 4 2	3 8 4	4 0 1	4 5 3	3 7 1	4 0 3	4 0 4	4 5 4	3 9 1	4 1 3	4 2 2	4 4 5	3 9 4
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Squamous cell papilloma																									
Prostate	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma								X		X				X	X	X		X	X		X	X	X		X
Interstitial cell, adenoma			X			X	X					X	X				X			X				X	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																				X					
Lymph node				+					+				+		+	+		+	+		+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma													X												
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma																						X			
Histiocytic sarcoma																				X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma											X						X								
Squamous cell papilloma																					X				
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma																				X					
Subcutaneous tissue, fibrosarcoma																		X							
Subcutaneous tissue, histiocytic sarcoma																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																				+					
Histiocytic sarcoma																				X					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Histiocytic sarcoma																				X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	4 6 2	3 7 2	3 7 4	3 8 2	3 8 3	3 8 5	3 9 3	3 9 5	4 0 2	4 1 1	4 1 2	4 1 4	4 1 5	4 2 1	4 2 3	4 3 1	4 3 3	4 3 4	4 3 5	4 4 1	4 5 1	4 5 2	4 5 5	4 6 4	4 6 5	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																X										2
Squamous cell papilloma							X																			1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	33
Interstitial cell, adenoma					X				X										X							11
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							2
Lymph node		+			+								+		+					+				+		18
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenocarcinoma										X																1
Fibroadenoma																										1
Histiocytic sarcoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma												X														3
Squamous cell papilloma																										1
Sebaceous gland, adenoma								X																		1
Subcutaneous tissue, fibroma																						X				2
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, histiocytic sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Histiocytic sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X				X												2
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	3	4	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7
	0	5	6	0	0	3	3	6	8	8	0	1	1	4	4	6	8	8	8	9	9	0	0	2
	5	0	0	6	6	1	2	6	0	3	1	0	4	6	6	8	0	0	4	2	9	2	2	2
Carcass ID Number	3	4	3	3	4	4	4	3	4	4	4	4	4	3	4	4	3	4	4	4	3	4	4	4
	8	6	9	7	6	4	2	7	4	0	2	3	4	8	0	5	7	0	0	5	9	1	2	4
	1	1	2	3	3	3	4	5	4	5	5	2	2	4	1	3	1	3	4	4	1	3	2	5
Special Senses System																								
Eye																				+				
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Carcinoma										X														
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra															+									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																				X				
Leukemia mononuclear		X			X		X	X	X		X			X	X	X	X	X			X		X	X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	4 6 2	3 7 2	3 7 4	3 8 2	3 8 3	3 8 5	3 9 3	3 9 5	4 0 2	4 1 1	4 1 2	4 1 4	4 1 5	4 2 1	4 2 3	4 3 1	4 3 3	4 3 4	4 3 5	4 4 1	4 5 1	4 5 2	4 5 5	4 6 4	4 6 5	Total Tissues/ Tumors
Special Senses System																										
Eye									+																	2
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							2
Leukemia mononuclear		X				X						X	X	X	X		X				X	X	X	X		26

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	15/50 (30%)	9/50 (18%)	12/50 (24%)	12/50 (24%)
Adjusted rates[b]	43.7%	27.4%	37.3%	40.6%
Terminal rates[c]	11/30 (37%)	4/25 (16%)	9/29 (31%)	8/24 (33%)
First incidence (days)	630	614	694	614
Life table tests[d]	P=0.445	P=0.207N	P=0.333N	P=0.522N
Logistic regression tests[d]	P=0.521	P=0.092N	P=0.217N	P=0.372N
Cochran-Armitage test[d]	P=0.508N			
Fisher exact test[d]		P=0.121N	P=0.326N	P=0.326N
Liver: Hepatocellular Adenoma				
Overall rates	5/50 (10%)	6/50 (12%)	2/50 (4%)	6/50 (12%)
Adjusted rates	16.7%	19.2%	6.9%	21.3%
Terminal rates	5/30 (17%)	3/25 (12%)	2/29 (7%)	4/24 (17%)
First incidence (days)	729 (T)	617	729 (T)	601
Life table tests	P=0.349	P=0.419	P=0.226N	P=0.372
Logistic regression tests	P=0.400	P=0.528	P=0.226N	P=0.462
Cochran-Armitage test	P=0.451			
Fisher exact test		P=0.500	P=0.218N	P=0.500
Mammary Gland: Fibroadenoma				
Overall rates	2/50 (4%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rates	6.4%	12.0%	2.6%	3.4%
Terminal rates	1/30 (3%)	3/25 (12%)	0/29 (0%)	0/24 (0%)
First incidence (days)	693	729 (T)	680	702
Life table tests	P=0.349N	P=0.431	P=0.481N	P=0.541N
Logistic regression tests	P=0.335N	P=0.497	P=0.482N	P=0.516N
Cochran-Armitage test	P=0.311N			
Fisher exact test		P=0.500	P=0.500N	P=0.500N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rates	2/50 (4%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rates	6.4%	12.0%	2.6%	7.5%
Terminal rates	1/30 (3%)	3/25 (12%)	0/29 (0%)	1/24 (4%)
First incidence (days)	693	729 (T)	680	702
Life table tests	P=0.606N	P=0.431	P=0.481N	P=0.640
Logistic regression tests	P=0.593N	P=0.497	P=0.482N	P=0.677
Cochran-Armitage test	P=0.555N			
Fisher exact test		P=0.500	P=0.500N	P=0.691N
Pancreatic Islets: Adenoma				
Overall rates	4/50 (8%)	5/50 (10%)	3/50 (6%)	7/50 (14%)
Adjusted rates	12.3%	16.4%	10.3%	25.3%
Terminal rates	3/30 (10%)	2/25 (8%)	3/29 (10%)	5/24 (21%)
First incidence (days)	618	637	729 (T)	531
Life table tests	P=0.125	P=0.452	P=0.503N	P=0.172
Logistic regression tests	P=0.159	P=0.526	P=0.453N	P=0.242
Cochran-Armitage test	P=0.194			
Fisher exact test		P=0.500	P=0.500N	P=0.262

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Pancreatic Islets: Adenoma or Carcinoma				
Overall rates	4/50 (8%)	5/50 (10%)	4/50 (8%)	7/50 (14%)
Adjusted rates	12.3%	16.4%	13.2%	25.3%
Terminal rates	3/30 (10%)	2/25 (8%)	3/29 (10%)	5/24 (21%)
First incidence (days)	618	637	714	531
Life table tests	P=0.135	P=0.452	P=0.640	P=0.172
Logistic regression tests	P=0.170	P=0.526	P=0.596N	P=0.242
Cochran-Armitage test	P=0.208			
Fisher exact test		P=0.500	P=0.643N	P=0.262
Pharynx: Squamous Cell Papilloma				
Overall rates	0/50 (0%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted rates	0.0%	0.0%	0.0%	19.3%
Terminal rates	0/30 (0%)	0/25 (0%)	0/29 (0%)	4/24 (17%)
First incidence (days)	–[e]	–	–	684
Life table tests	P<0.001	–	–	P=0.020
Logistic regression tests	P<0.001	–	–	P=0.026
Cochran-Armitage test	P<0.001			
Fisher exact test		–	–	P=0.028
Pharynx: Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted rates	0.0%	0.0%	0.0%	23.3%
Terminal rates	0/30 (0%)	0/25 (0%)	0/29 (0%)	5/24 (21%)
First incidence (days)	–	–	–	684
Life table tests	P<0.001	–	–	P=0.009
Logistic regression tests	P<0.001	–	–	P=0.013
Cochran-Armitage test	P<0.001			
Fisher exact test		–	–	P=0.013
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	15/50 (30%)	14/50 (28%)	16/48 (33%)	12/50 (24%)
Adjusted rates	42.1%	38.6%	45.6%	40.9%
Terminal rates	10/30 (33%)	5/25 (20%)	10/28 (36%)	8/24 (33%)
First incidence (days)	572	369	537	601
Life table tests	P=0.451N	P=0.562	P=0.487	P=0.516N
Logistic regression tests	P=0.334N	P=0.474N	P=0.533	P=0.363N
Cochran-Armitage test	P=0.284N			
Fisher exact test		P=0.500N	P=0.445	P=0.326N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rates	17/50 (34%)	14/50 (28%)	16/48 (33%)	12/50 (24%)
Adjusted rates	44.5%	38.6%	45.6%	40.9%
Terminal rates	10/30 (33%)	5/25 (20%)	10/28 (36%)	8/24 (33%)
First incidence (days)	506	369	537	601
Life table tests	P=0.354N	P=0.438N	P=0.512N	P=0.357N
Logistic regression tests	P=0.226N	P=0.340N	P=0.515N	P=0.205N
Cochran-Armitage test	P=0.200N			
Fisher exact test		P=0.333N	P=0.557N	P=0.189N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Preputial Gland: Adenoma				
Overall rates	2/50 (4%)	3/50 (6%)	2/50 (4%)	2/49 (4%)
Adjusted rates	6.7%	6.7%	6.1%	6.2%
Terminal rates	2/30 (7%)	0/25 (0%)	1/29 (3%)	1/24 (4%)
First incidence (days)	729 (T)	618	686	506
Life table tests	P=0.586N	P=0.514	P=0.690N	P=0.631
Logistic regression tests	P=0.528N	P=0.485	P=0.663N	P=0.687
Cochran-Armitage test	P=0.545N			
Fisher exact test		P=0.500	P=0.691N	P=0.684
Skin: Keratoacanthoma				
Overall rates	1/50 (2%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rates	3.3%	11.0%	5.6%	9.3%
Terminal rates	1/30 (3%)	2/25 (8%)	1/29 (3%)	1/24 (4%)
First incidence (days)	729 (T)	713	632	601
Life table tests	P=0.306	P=0.259	P=0.518	P=0.269
Logistic regression tests	P=0.343	P=0.290	P=0.514	P=0.299
Cochran-Armitage test	P=0.363			
Fisher exact test		P=0.309	P=0.500	P=0.309
Skin: Basal Cell Adenoma or Basal Cell Carcinoma				
Overall rates	2/50 (4%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rates	6.7%	8.5%	0.0%	0.0%
Terminal rates	2/30 (7%)	1/25 (4%)	0/29 (0%)	0/24 (0%)
First incidence (days)	729 (T)	621	–	–
Life table tests	P=0.116N	P=0.473	P=0.245N	P=0.288N
Logistic regression tests	P=0.103N	P=0.511	P=0.245N	P=0.288N
Cochran-Armitage test	P=0.101N			
Fisher exact test		P=0.500	P=0.247N	P=0.247N
Skin: Keratoacanthoma, Squamous Cell Papilloma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rates	4/50 (8%)	6/50 (12%)	2/50 (4%)	4/50 (8%)
Adjusted rates	13.3%	18.9%	5.6%	12.3%
Terminal rates	4/30 (13%)	3/25 (12%)	1/29 (3%)	1/24 (4%)
First incidence (days)	729 (T)	621	632	601
Life table tests	P=0.567N	P=0.309	P=0.333N	P=0.562
Logistic regression tests	P=0.514N	P=0.395	P=0.297N	P=0.624
Cochran-Armitage test	P=0.487N			
Fisher exact test		P=0.370	P=0.339N	P=0.643N
Skin (Subcutaneous Tissue): Fibroma				
Overall rates	4/50 (8%)	4/50 (8%)	5/50 (10%)	2/50 (4%)
Adjusted rates	12.3%	15.3%	15.2%	7.3%
Terminal rates	3/30 (10%)	3/25 (12%)	3/29 (10%)	1/24 (4%)
First incidence (days)	618	721	686	692
Life table tests	P=0.309N	P=0.571	P=0.515	P=0.415N
Logistic regression tests	P=0.278N	P=0.627N	P=0.547	P=0.354N
Cochran-Armitage test	P=0.246N			
Fisher exact test		P=0.643N	P=0.500	P=0.339N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Skin (Subcutaneous Tissue): Fibroma, Neurofibrosarcoma, Fibrosarcoma, or Sarcoma				
Overall rates	5/50 (10%)	4/50 (8%)	5/50 (10%)	3/50 (6%)
Adjusted rates	14.3%	15.3%	15.2%	10.0%
Terminal rates	3/30 (10%)	3/25 (12%)	3/29 (10%)	1/24 (4%)
First incidence (days)	572	721	686	680
Life table tests	P=0.397N	P=0.563N	P=0.609N	P=0.428N
Logistic regression tests	P=0.356N	P=0.478N	P=0.611N	P=0.364N
Cochran-Armitage test	P=0.325N			
Fisher exact test		P=0.500N	P=0.630N	P=0.357N
Testes: Adenoma				
Overall rates	45/50 (90%)	46/50 (92%)	48/50 (96%)	44/50 (88%)
Adjusted rates	100.0%	97.8%	98.0%	100.0%
Terminal rates	30/30 (100%)	24/25 (96%)	28/29 (97%)	24/24 (100%)
First incidence (days)	539	601	533	460
Life table tests	P=0.271	P=0.267	P=0.426	P=0.209
Logistic regression tests	P=0.538	P=0.450N	P=0.451	P=0.641
Cochran-Armitage test	P=0.325N			
Fisher exact test		P=0.500	P=0.218	P=0.500N
Thyroid Gland (C-cell): Adenoma				
Overall rates	7/50 (14%)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rates	19.1%	4.0%	11.6%	15.4%
Terminal rates	3/30 (10%)	1/25 (4%)	2/29 (7%)	3/24 (13%)
First incidence (days)	548	729 (T)	581	699
Life table tests	P=0.593	P=0.047N	P=0.256N	P=0.360N
Logistic regression tests	P=0.530N	P=0.033N	P=0.273N	P=0.273N
Cochran-Armitage test	P=0.513N			
Fisher exact test		P=0.030N	P=0.262N	P=0.262N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rates	8/50 (16%)	1/50 (2%)	5/50 (10%)	5/50 (10%)
Adjusted rates	22.1%	4.0%	14.2%	19.4%
Terminal rates	4/30 (13%)	1/25 (4%)	2/29 (7%)	4/24 (17%)
First incidence (days)	548	729 (T)	581	699
Life table tests	P=0.510	P=0.029N	P=0.271N	P=0.393N
Logistic regression tests	P=0.573	P=0.017N	P=0.278N	P=0.293N
Cochran-Armitage test	P=0.573N			
Fisher exact test		P=0.015N	P=0.277N	P=0.277N
All Organs: Mononuclear Cell Leukemia				
Overall rates	18/50 (36%)	25/50 (50%)	23/50 (46%)	26/50 (52%)
Adjusted rates	47.3%	59.3%	57.2%	64.7%
Terminal rates	11/30 (37%)	9/25 (36%)	13/29 (45%)	11/24 (46%)
First incidence (days)	548	614	537	450
Life table tests	P=0.086	P=0.113	P=0.266	P=0.048
Logistic regression tests	P=0.140	P=0.124	P=0.235	P=0.074
Cochran-Armitage test	P=0.150			
Fisher exact test		P=0.113	P=0.208	P=0.079

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
All Organs: Benign Neoplasms				
Overall rates	47/50 (94%)	50/50 (100%)	49/50 (98%)	46/50 (92%)
Adjusted rates	100.0%	100.0%	100.0%	100.0%
Terminal rates	30/30 (100%)	25/25 (100%)	29/29 (100%)	24/24 (100%)
First incidence (days)	539	369	533	460
Life table tests	P=0.308	P=0.182	P=0.498	P=0.217
Logistic regression tests	P=0.328N	P=0.160	P=0.309	P=0.723
Cochran-Armitage test	P=0.144N			
Fisher exact test		P=0.121	P=0.309	P=0.500N
All Organs: Malignant Neoplasms				
Overall rates	27/50 (54%)	27/50 (54%)	28/50 (56%)	31/50 (62%)
Adjusted rates	60.2%	61.0%	63.8%	72.6%
Terminal rates	13/30 (43%)	9/25 (36%)	14/29 (48%)	13/24 (54%)
First incidence (days)	438	601	533	450
Life table tests	P=0.127	P=0.499	P=0.555	P=0.169
Logistic regression tests	P=0.239	P=0.519	P=0.431	P=0.281
Cochran-Armitage test	P=0.211			
Fisher exact test		P=0.579N	P=0.500	P=0.272
All Organs: Benign or Malignant Neoplasms				
Overall rates	49/50 (98%)	50/50 (100%)	49/50 (98%)	49/50 (98%)
Adjusted rates	100.0%	100.0%	100.0%	100.0%
Terminal rates	30/30 (100%)	25/25 (100%)	29/29 (100%)	24/24 (100%)
First incidence (days)	438	369	533	450
Life table tests	P=0.200	P=0.280	P=0.501N	P=0.187
Logistic regression tests	–[f]	–	–	–
Cochran-Armitage test	P=0.559N			
Fisher exact test		P=0.500	P=0.753N	P=0.753N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed

TABLE A4a
Historical Incidence of Oral Epithelium Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	0/50	0/50	0/50
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	10/1,253 (0.8%)	0/1,253 (0.0%)	10/1,253 (0.8%)
Standard deviation	1.4%		1.4%
Range	0%-4%		0%-4%

[a] Data as of 20 August 1992

TABLE A4b
Historical Incidence of Forestomach Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	0/50	0/50	0/50
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	3/1,253 (0.2%)	1/1,253 (0.1%)	4/1,253 (0.3%)
Standard deviation	0.6%	0.4%	0.8%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 20 August 1992

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	9
Early deaths				
Moribund	13	22	16	18
Natural deaths	7	3	5	9
Survivors				
Terminal sacrifice	30	25	29	24
Animals examined microscopically	60	60	60	59
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(9)
Angiectasis			1 (10%)	
Basophilic focus	4 (40%)	4 (40%)	6 (60%)	4 (44%)
Clear cell focus			4 (40%)	5 (56%)
Congestion	2 (20%)	1 (10%)		
Degeneration, cystic			2 (20%)	1 (11%)
Developmental malformation		1 (10%)	1 (10%)	1 (11%)
Inflammation, chronic				1 (11%)
Mineralization, capsule			1 (10%)	
Mixed cell focus	1 (10%)			2 (22%)
Necrosis			1 (10%)	
Pancreas	(10)	(10)	(10)	(9)
Atrophy, acinar cell	9 (90%)	1 (10%)		4 (44%)
Stomach, forestomach	(10)	(10)	(10)	(9)
Hyperplasia, basal cell, focal				1 (11%)
Cardiovascular System				
None				
Endocrine System				
Adrenal gland, cortex	(10)	(10)	(10)	(9)
Vacuolization, cytoplasmic			1 (10%)	
Hyperplasia	1 (10%)			
Hypertrophy	1 (10%)			
Adrenal gland, medulla	(10)	(10)	(10)	(9)
Hyperplasia	1 (10%)	1 (10%)		2 (22%)
Islets, pancreatic	(10)	(10)	(10)	(9)
Hyperplasia, focal	1 (10%)			
Pituitary	(9)	(10)	(10)	(9)
Pars distalis, hyperplasia	3 (33%)	4 (40%)	2 (20%)	2 (22%)
Pars distalis, angiectasis	1 (11%)		1 (10%)	
Thyroid gland	(10)	(10)	(10)	(9)
Follicle, dilatation				1 (11%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
General Body System				
None				
Genital System				
Preputial gland	(10)	(10)	(10)	(9)
Duct, dilatation	1 (10%)			
Hyperplasia		1 (10%)		
Prostate	(10)	(10)	(10)	(9)
Inflammation	1 (10%)		1 (10%)	1 (11%)
Testes	(10)	(10)	(10)	(9)
Interstitial cell, hyperplasia	5 (50%)	7 (70%)	6 (60%)	4 (44%)
Hematopoietic System				
Lymph node	(10)	(10)	(10)	(9)
Mediastinal, congestion	1 (10%)	1 (10%)		
Pancreatic, hyperplasia	1 (10%)			
Lymph node, mandibular	(10)	(10)	(10)	(9)
Congestion	5 (50%)	4 (40%)	6 (60%)	4 (44%)
Hyperplasia, plasma cell		1 (10%)		1 (11%)
Lymph node, mesenteric	(10)	(10)	(10)	(9)
Congestion		1 (10%)		
Thymus	(9)	(10)	(9)	(9)
Hemorrhage	1 (11%)			2 (22%)
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(9)
Congestion	4 (40%)		1 (10%)	2 (22%)
Hemmorrhage	1 (10%)		1 (10%)	2 (22%)
Interstitium, inflammation,	1 (10%)	1 (10%)		1 (11%)
Infiltration cellular, histocyte	1 (10%)			
Alveolar epithelium, hyperplasia		1 (10%)		
Artery, mineralization				1 (11%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Respiratory System (continued)				
Nose	(10)	(10)	(10)	(9)
Foreign body, lumen	2 (20%)	4 (40%)		
Fungus			3 (30%)	
Inflammation, acute	9 (90%)	5 (50%)	1 (10%)	3 (33%)
Inflammation, chronic active		2 (20%)	3 (30%)	
Special Senses System				
None				
Urinary System				
Kidney	(10)	(10)	(10)	(9)
Nephropathy	9 (90%)	9 (90%)	9 (90%)	9 (100%)
2-Year Study				
Alimentary System				
Intestine large, cecum	(46)	(49)	(49)	(49)
Hyperplasia, focal, lymphoid			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal		2 (4%)	7 (14%)	2 (4%)
Basophilic focus	30 (60%)	26 (52%)	34 (68%)	18 (36%)
Clear cell focus	13 (26%)	13 (26%)	19 (38%)	17 (34%)
Congestion	3 (6%)	4 (8%)	4 (8%)	1 (2%)
Degeneration, cystic, focal	15 (30%)	13 (26%)	18 (36%)	25 (50%)
Developmental malformation	2 (4%)	1 (2%)		
Eosinophilic focus	9 (18%)	11 (22%)	15 (30%)	20 (40%)
Fatty change, diffuse	1 (2%)	5 (10%)	1 (2%)	
Fatty change, focal	11 (22%)	11 (22%)	12 (24%)	16 (32%)
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule				1 (2%)
Hyperplasia, focal			1 (2%)	
Infiltration cellular, focal, lymphocyte	1 (2%)			
Inflammation, chronic, focal	6 (12%)	2 (4%)	5 (10%)	2 (4%)
Mixed cell focus	4 (8%)	1 (2%)		7 (14%)
Necrosis, focal	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Pigmentation, focal	1 (2%)			1 (2%)
Bile duct, cyst	1 (2%)		2 (4%)	1 (2%)
Bile duct, fibrosis				1 (2%)
Bile duct, hyperplasia, focal	6 (12%)	3 (6%)	9 (18%)	2 (4%)
Centrilobular, atrophy, focal				1 (2%)
Centrilobular, necrosis	1 (2%)			
Vein, dilatation				1 (2%)
Mesentery	(5)	(5)	(7)	(4)
Fat, necrosis, focal	4 (80%)	5 (100%)	5 (71%)	3 (75%)
Pancreas	(50)	(50)	(50)	(50)
Accessory spleen		1 (2%)		
Inflammation, acute, focal			1 (2%)	
Acinus, atrophy, diffuse	1 (2%)	1 (2%)	1 (2%)	1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Pancreas (continued)				
Acinus, atrophy, focal	28 (56%)	26 (52%)	29 (58%)	26 (52%)
Acinus, cytoplasmic alteration, focal				1 (2%)
Acinus, hyperplasia, focal			1 (2%)	
Acinus, vacuolization cytoplasmic, focal	1 (2%)			
Artery, inflammation, focal	1 (2%)		1 (2%)	
Pharynx		(1)		(9)
Palate, hyperplasia, focal, squamous				2 (22%)
Palate, inflammation, chronic active		1 (100%)		
Salivary glands	(50)	(50)	(50)	(50)
Atrophy, focal	1 (2%)			
Hyperplasia, focal				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Acanthosis, focal		1 (2%)		1 (2%)
Hyperplasia, basal cell, focal		2 (4%)	10 (20%)	19 (38%)
Hyperplasia, focal, plasma cell				1 (2%)
Hyperplasia, focal, squamous	1 (2%)	1 (2%)	6 (12%)	4 (8%)
Inflammation, chronic, focal	1 (2%)	1 (2%)		
Inflammation, chronic active, focal		2 (4%)	2 (4%)	
Ulcer, focal	4 (8%)	3 (6%)	3 (6%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Erosion, focal		2 (4%)		1 (2%)
Hyperplasia, basal cell, focal			1 (2%)	
Inflammation, acute, focal		1 (2%)		
Ulcer, focal		3 (6%)		
Tooth		(1)	(1)	
Gingiva, inflammation, chronic active		1 (100%)		
Peridontal tissue, inflammation, focal			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	37 (74%)	31 (62%)	38 (76%)	32 (64%)
Mineralization, focal	1 (2%)		1 (2%)	1 (2%)
Atrium, dilatation	2 (4%)	3 (6%)	3 (6%)	
Atrium, thrombosis	6 (12%)	2 (4%)	1 (2%)	1 (2%)
Valve, degeneration	1 (2%)			
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Angiectasis, focal		1 (2%)		
Congestion		1 (2%)		1 (2%)
Cyst		1 (2%)		
Cyst, multiple				1 (2%)
Degeneration, cystic, focal		2 (4%)		
Hemorrhage	1 (2%)			1 (2%)
Hyperplasia, focal	6 (12%)	6 (12%)	6 (12%)	2 (4%)
Vacuolization cytoplasmic, focal	8 (16%)	12 (24%)	3 (6%)	12 (24%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Endocrine System (continued)				
Adrenal medulla	(49)	(50)	(50)	(50)
Cyst			1 (2%)	
Hyperplasia	16 (33%)	12 (24%)	14 (28%)	8 (16%)
Infiltration cellular, focal, lymphocyte	1 (2%)			
Bilateral, hyperplasia	4 (8%)	3 (6%)	3 (6%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia, focal	5 (10%)	4 (8%)	7 (14%)	10 (20%)
Parathyroid gland	(43)	(47)	(46)	(45)
Hyperplasia	1 (2%)		2 (4%)	
Bilateral, hyperplasia	1 (2%)			2 (4%)
Pituitary gland	(50)	(50)	(48)	(50)
Angiectasis, focal	1 (2%)	1 (2%)		
Pigmentation, focal		1 (2%)		
Pars distalis, cyst	3 (6%)	2 (4%)	3 (6%)	
Pars distalis, hyperplasia, focal	12 (24%)	16 (32%)	11 (23%)	18 (36%)
Pars distalis, hypertrophy, focal	1 (2%)			
Pars nervosa, hyperplasia, focal				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia, focal	7 (14%)	6 (12%)	10 (20%)	4 (8%)
Follicle, dilatation, focal				4 (8%)
Follicular cell, hyperplasia, focal			1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Degeneration, focal	31 (62%)	38 (76%)	37 (74%)	33 (66%)
Preputial gland	(50)	(50)	(50)	(49)
Hyperplasia	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic				1 (2%)
Inflammation, suppurative	5 (10%)	3 (6%)	2 (4%)	1 (2%)
Duct, dilatation	10 (20%)	17 (34%)	9 (18%)	11 (22%)
Prostate	(50)	(50)	(50)	(49)
Abscess		1 (2%)		2 (4%)
Atrophy	10 (20%)	7 (14%)	11 (22%)	13 (27%)
Degeneration, focal	1 (2%)	1 (2%)		
Hyperplasia, focal	3 (6%)		3 (6%)	
Inflammation, chronic, focal	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Inflammation, focal, granulomatous			3 (6%)	2 (4%)
Inflammation, focal, suppurative	3 (6%)	2 (4%)	4 (8%)	4 (8%)
Inflammation, suppurative	3 (6%)			
Vacuolization cytoplasmic, focal	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(49)
Abscess				1 (2%)
Dilatation			1 (2%)	
Hyperplasia, focal		1 (2%)		
Inflammation, chronic, focal				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Genital System (continued)				
Testes	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	3 (6%)	4 (8%)	9 (18%)
Cyst			2 (4%)	
Cyst, multiple	1 (2%)			
Mineralization, focal		1 (2%)		
Artery, inflammation, focal			1 (2%)	
Bilateral, atrophy		2 (4%)		
Interstitial cell, hyperplasia, focal	4 (8%)	2 (4%)	1 (2%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	1 (2%)		3 (6%)	4 (8%)
Lymph node	(15)	(16)	(23)	(18)
Axillary, hyperplasia, plasma cell				1 (6%)
Bronchial, hemorrhage	1 (7%)			
Bronchial, infiltration cellular, histiocyte		1 (6%)		
Iliac, cyst				1 (6%)
Iliac, infiltration cellular, histiocyte				1 (6%)
Inguinal, cyst				1 (6%)
Lumbar, hemorrhage			1 (4%)	2 (11%)
Lumbar, hyperplasia, plasma cell				1 (6%)
Lumbar, infiltration cellular, histiocyte			2 (9%)	
Mediastinal, cyst			1 (4%)	
Mediastinal, hemorrhage	5 (33%)	2 (13%)	5 (22%)	2 (11%)
Mediastinal, hyperplasia, plasma cell	2 (13%)		1 (4%)	
Mediastinal, infiltration cellular, histiocyte			1 (4%)	1 (6%)
Pancreatic, hemorrhage			1 (4%)	2 (11%)
Pancreatic, infiltration cellular, histiocyte			1 (4%)	
Renal, hemorrhage	1 (7%)	1 (6%)	1 (4%)	1 (6%)
Renal, infiltration cellular, histiocyte				1 (6%)
Lymph node, mandibular	(48)	(50)	(49)	(50)
Cyst	3 (6%)	2 (4%)	6 (12%)	3 (6%)
Hemorrhage	3 (6%)	2 (4%)	3 (6%)	2 (4%)
Hyperplasia, plasma cell	9 (19%)	10 (20%)	4 (8%)	3 (6%)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Cyst		1 (2%)		
Edema			1 (2%)	
Hemorrhage	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Hyperplasia, lymphoid	1 (2%)			
Infiltration cellular, histiocyte	1 (2%)			
Spleen	(50)	(50)	(50)	(49)
Congestion	1 (2%)		1 (2%)	
Depletion cellular		1 (2%)		
Fibrosis, focal	2 (4%)	1 (2%)	4 (8%)	4 (8%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)		2 (4%)
Pigmentation, hemosiderin	1 (2%)			
Capsule, cyst				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Thymus	(48)	(40)	(49)	(48)
Congestion				1 (2%)
Hemorrhage	1 (2%)	1 (3%)		1 (2%)
Integumentary System				
Mammary gland	(48)	(49)	(50)	(46)
Galactocele	6 (13%)	7 (14%)	1 (2%)	1 (2%)
Hyperplasia, focal	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Inflammation, acute, focal		1 (2%)		
Inflammation, chronic, focal				1 (2%)
Pigmentation, focal				1 (2%)
Skin	(50)	(50)	(50)	(50)
Acanthosis, focal			1 (2%)	
Subcutaneous tissue, inflammation, acute				1 (2%)
Subcutaneous tissue, inflammation, chronic active, focal	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy			1 (2%)	1 (2%)
Hyperostosis	1 (2%)			
Hyperplasia, focal				1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis, focal				1 (2%)
Hemorrhage, focal	1 (2%)			
Hydrocephalus	4 (8%)	4 (8%)	1 (2%)	1 (2%)
Infiltration cellular, focal, lymphocyte	1 (2%)			
Mineralization, focal			1 (2%)	
Necrosis, focal	1 (2%)	1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	6 (12%)	1 (2%)	1 (2%)	3 (6%)
Hemorrhage, focal				1 (2%)
Infiltration cellular, focal, lymphocyte		3 (6%)	3 (6%)	
Infiltration cellular, focal, histiocyte	6 (12%)	4 (8%)	6 (12%)	4 (8%)
Inflammation, focal	7 (14%)	2 (4%)	7 (14%)	7 (14%)
Alveolar epithelium, hyperplasia, focal	2 (4%)	1 (2%)	3 (6%)	2 (4%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Respiratory System (continued)				
Nose	(50)	(50)	(49)	(50)
Congestion	1 (2%)		1 (2%)	
Foreign body	5 (10%)	2 (4%)	3 (6%)	3 (6%)
Fungus	7 (14%)	6 (12%)	16 (33%)	11 (22%)
Hemorrhage	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Inflammation, acute	7 (14%)	2 (4%)	1 (2%)	
Inflammation, chronic active	9 (18%)	10 (20%)	20 (41%)	14 (28%)
Inflammation, subacute	5 (10%)	1 (2%)	3 (6%)	3 (6%)
Glands, dilatation, focal				1 (2%)
Olfactory epithelium, necrosis, focal		1 (2%)		
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic active, focal			1 (2%)	
Special Senses System				
Eye	(5)	(4)	(3)	(2)
Inflammation, chronic		1 (25%)		
Phthisis bulbi	1 (20%)	1 (25%)		
Cornea, degeneration, focal			1 (33%)	
Cornea, inflammation	1 (20%)			
Lens, cataract	3 (60%)	1 (25%)	1 (33%)	1 (50%)
Lens, inflammation, focal, subacute	1 (20%)			
Retina, atrophy	1 (20%)	2 (50%)		1 (50%)
Zymbal's gland	(48)	(48)	(48)	(48)
Hyperplasia	1 (2%)			
Inflammation, chronic active				1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst			2 (4%)	2 (4%)
Hydronephrosis			1 (2%)	
Infarct				1 (2%)
Mineralization, focal				1 (2%)
Nephropathy	48 (96%)	50 (100%)	50 (100%)	46 (92%)
Pigmentation, focal	12 (24%)	11 (22%)	15 (30%)	23 (46%)
Pelvis, inflammation, suppurative				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Abscess				1 (2%)

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF C.I. DIRECT BLUE 218

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	9	9	10	10
Early deaths				
Moribund	12	15	13	16
Natural deaths	4	7	6	9
Survivors				
Died last week of study		1		
Terminal sacrifice	35	28	31	25
Animals examined microscopically	59	59	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(9)	(9)	(10)	(10)
Leukemia mononuclear				1 (10%)
Cardiovascular System				
None				
Endocrine System				
Pituitary gland	(9)	(8)	(10)	(10)
Pars distalis, adenoma	1 (11%)	1 (13%)	2 (20%)	2 (20%)
Thyroid gland	(9)	(8)	(10)	(10)
C-cell, adenoma bilateral	1 (11%)			
General Body System				
None				
Genital System				
Uterus	(9)	(9)	(10)	(10)
Polyp stromal	1 (11%)	1 (11%)	1 (10%)	2 (20%)
Hematopoietic System				
Spleen	(9)	(9)	(10)	(10)
Leukemia mononuclear				1 (10%)
Integumentary System				
Mammary gland	(9)	(9)	(9)	(10)
Fibroadenoma	1 (11%)			
Musculoskeletal System				
None				

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218[a] (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Nervous System				
None				
Respiratory System				
None				
Special Senses System				
None				
Urinary System				
None				
Systemic Lesions				
None				
2-Year Study				
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Lipoma		1 (2%)		
Intestine large, colon	(49)	(47)	(49)	(48)
Intestine large, rectum	(48)	(47)	(49)	(48)
Intestine large, cecum	(48)	(47)	(48)	(48)
Intestine small, duodenum	(48)	(48)	(50)	(45)
Intestine small, jejunum	(48)	(45)	(49)	(47)
Carcinoma			1 (2%)	
Intestine small, ileum	(48)	(46)	(48)	(46)
Liver	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Mesentery	(8)	(6)	(3)	(6)
Sarcoma, metastatic, skin		1 (17%)		
Schwannoma malignant, metastatic, uterus		1 (17%)		
Fat, sarcoma			1 (33%)	
Pancreas	(50)	(48)	(50)	(48)
Pharynx	(1)	(1)		(2)
Palate, squamous cell papilloma	1 (100%)	1 (100%)		2 (100%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(50)	(49)
Squamous cell papilloma				1 (2%)
Stomach, glandular	(50)	(48)	(50)	(48)
Sarcoma, metastatic, skin		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma NOS		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Carcinoma, metastatic, thyroid gland				1 (2%)
Adrenal medulla	(49)	(50)	(47)	(49)
Pheochromocytoma benign	1 (2%)			2 (4%)
Bilateral, pheochromocytoma benign			1 (2%)	1 (2%)
Islets, pancreatic	(50)	(48)	(50)	(48)
Adenoma		1 (2%)	2 (4%)	
Parathyroid gland	(45)	(43)	(39)	(44)
Pituitary gland	(50)	(47)	(49)	(47)
Pars distalis, adenoma	26 (52%)	29 (62%)	30 (61%)	20 (43%)
Pars distalis, adenoma, multiple	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Pars distalis, carcinoma	2 (4%)		1 (2%)	2 (4%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(49)	(49)
Bilateral, C-cell, adenoma	1 (2%)			1 (2%)
C-cell, adenoma	2 (4%)	4 (8%)	2 (4%)	2 (4%)
C-cell, carcinoma	1 (2%)			3 (6%)
Follicular cell, adenoma			2 (4%)	
General Body System				
Tissue NOS		(1)		
Schwannoma malignant, metastatic, uterus		1 (100%)		
Genital System				
Clitoral gland	(47)	(48)	(49)	(47)
Adenoma	2 (4%)		2 (4%)	2 (4%)
Carcinoma		1 (2%)		
Squamous cell carcinoma				1 (2%)
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign		1 (2%)		
Sarcoma, metastatic, skin		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Leiomyosarcoma			1 (2%)	
Polyp stromal	1 (2%)	12 (24%)	10 (20%)	10 (20%)
Sarcoma		1 (2%)		
Schwannoma malignant		1 (2%)		
Cervix, schwannoma malignant, metastatic, uterus		1 (2%)		
Vagina	(1)	(1)	(2)	(3)
Fibroma				1 (33%)
Leiomyosarcoma	1 (100%)			
Schwannoma malignant, metastatic, uterus		1 (100%)		
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(49)
Lymph node	(7)	(12)	(6)	(14)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node, mandibular	(49)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland				1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Schwannoma malignant, metastatic, uterus		1 (2%)		
Spleen	(50)	(49)	(50)	(48)
Thymus	(48)	(49)	(42)	(45)
Thymoma benign				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoacanthoma				1 (2%)
Adenocarcinoma	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Adenoma				1 (2%)
Fibroadenoma	14 (28%)	11 (22%)	16 (32%)	10 (20%)
Fibroadenoma, multiple	5 (10%)	1 (2%)	3 (6%)	
Sarcoma, metastatic, skin		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma		1 (2%)		
Subcutaneous tissue, fibroma				1 (2%)
Subcutaneous tissue, fibrosarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma		1 (2%)		
Musculoskeletal System				
Skeletal muscle	(1)			(1)
Back, sarcoma				1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma NOS			1 (2%)	
Carcinoma, metastatic, pituitary gland	1 (2%)			1 (2%)
Ependymoma benign			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		2 (4%)
Carcinoma, metastatic, thyroid gland	1 (2%)			2 (4%)
Fibrosarcoma, metastatic, ear			1 (2%)	
Sarcoma, metastatic, skin		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(49)	(50)
Carcinoma, metastatic, thyroid gland				2 (4%)
Special Senses System				
Ear			(1)	
Pinna, fibrosarcoma			1 (100%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Lipoma		1 (2%)		
Urinary bladder	(48)	(48)	(50)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	11 (22%)	16 (32%)	9 (18%)	16 (32%)
Lymphoma malignant lymphocytic		1 (2%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	46	46	45
Total primary neoplasms	74	92	89	84
Total animals with benign neoplasms	37	39	39	38
Total benign neoplasms	56	68	71	58
Total animals with malignant neoplasms	17	22	17	22
Total malignant neoplasms	18	23	17	26
Total animals with metastatic neoplasms	2	2	1	3
Total metastatic neoplasms	2	12	1	7
Total animals with uncertain neoplasms benign or malignant		1	1	
Total uncertain neoplasms		1	1	

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm

Number of Days on Study	2 7 0	4 2 8	4 6 0	4 9 9	5 3 2	5 3 2	6 4 2	6 5 9	6 6 6	6 6 6	6 7 1	6 7 1	6 7 3	6 8 4	7 1 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	5 2 4	5 3 1	5 7 2	5 6 5	4 9 3	4 9 5	5 0 1	5 2 2	5 3 3	5 8 4	4 9 4	5 4 4	4 9 1	5 4 3	5 8 3	5 9 2	5 0 2	5 0 3	5 0 4	5 0 5	5 1 1	5 1 2	5 1 3	5 1 4	5 1 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery												+	+		+		+								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Palate, squamous cell papilloma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																				X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X			X	X	X	X			X		X	X	X		X				X		X
Pars distalis, adenoma, multiple																									
Pars distalis, carcinoma						X					X														
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma																									
C-cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									X
Polyp stromal																			X						
Vagina					+																				
Leiomyosarcoma					X																				

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	5 2 1	5 2 3	5 2 5	5 3 2	5 3 4	5 3 5	5 4 1	5 4 2	5 4 5	5 5 1	5 5 2	5 5 3	5 5 4	5 5 5	5 6 1	5 6 2	5 6 3	5 6 4	5 7 1	5 7 3	5 7 4	5 7 5	5 8 1	5 8 2	5 8 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery				+	+							+								+						8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pharynx															+											1
Palate, squamous cell papilloma															X											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X			X	X		X			X	X		X		X		X	X	X	X		X		X	26
Pars distalis, adenoma, multiple				X																				X		2
Pars distalis, carcinoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma					X																					1
C-cell, adenoma	X																		X							2
C-cell, carcinoma												X														1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma					X		X																			2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Polyp stromal																										1
Vagina																										1
Leiomyosarcoma																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	2 7 0	4 2 8	4 6 0	4 9 9	5 3 2	5 3 2	6 4 2	6 5 9	6 6 6	6 6 6	6 7 1	6 7 1	6 7 3	6 8 4	7 1 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	5 2 4	5 3 1	5 7 2	5 6 5	4 9 3	4 9 5	5 0 1	5 2 2	5 3 3	5 8 4	4 9 4	5 4 4	4 9 1	5 4 3	5 8 3	5 9 2	5 0 2	5 0 3	5 0 4	5 0 5	5 1 1	5 1 2	5 1 3	5 1 4	5 1 5
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+							+	+		+	+	+	+										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma								X																	
Fibroadenoma									X		X						X	X			X	X	X		
Fibroadenoma, multiple													X						X						X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																	+								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland						X																			
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Zymbal's gland	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X					X		X		X		X		X		X							

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	5 2 1	5 2 3	5 2 5	5 3 2	5 3 4	5 3 5	5 4 1	5 4 2	5 4 5	5 5 1	5 5 2	5 5 3	5 5 4	5 5 5	5 6 1	5 6 2	5 6 3	5 6 4	5 7 1	5 7 3	5 7 4	5 7 5	5 8 1	5 8 2	5 8 5	Total Tissues/ Tumors
Hematopoletic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node																										7
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma			X					X																		3
Fibroadenoma		X					X			X	X			X	X						X					14
Fibroadenoma, multiple												X								X						5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pituitary gland																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland												X														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+										+															2
Zymbal's gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	45
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X				X												X									11

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm

Number of Days on Study	4 1 9	4 5 2	4 7 7	4 9 4	5 0 4	5 2 3	5 7 2	6 2 1	6 2 4	6 4 9	6 5 2	6 5 9	6 5 9	6 7 0	6 8 1	6 8 4	6 8 4	6 9 9	7 1 3	7 2 1	7 2 6	7 3 0	7 3 3	7 3 3	7 3 3
Carcass ID Number	6 4 5	6 1 1	6 2 5	6 8 3	7 0 5	6 5 4	7 0 4	6 5 3	7 0 3	6 5 2	6 6 5	6 2 1	6 9 3	6 3 2	6 4 4	6 9 1	7 0 2	6 8 1	6 8 4	6 6 3	6 8 2	6 3 5	6 1 2	6 1 3	6 1 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Intestine large, colon	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	A	A	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	A	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin		X																							
Mesentery		+								+	+														
Sarcoma, metastatic, skin		X																							
Schwannoma malignant, metastatic, uterus										X															
Pancreas	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Palate, squamous cell papilloma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin		X																							
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma NOS																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X		X		X	X			X					X	X		X	X	X	X	X		X	X
Pars distalis, adenoma, multiple					X																				
Pars intermedia, adenoma																	X								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
General Body System																									
Tissue NOS										+															
Schwannoma malignant, metastatic, uterus										X															

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	6 1 5	6 2 2	6 2 3	6 2 4	6 3 1	6 3 3	6 3 4	6 4 1	6 4 2	6 4 3	6 5 1	6 5 5	6 6 1	6 6 2	6 6 4	6 7 1	6 7 2	6 7 3	6 7 4	6 7 5	6 8 5	6 9 2	6 9 4	6 9 5	7 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma		X																								1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skin																										1
Mesentery									+										+					+		6
Sarcoma, metastatic, skin																										1
Schwannoma malignant, metastatic, uterus																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pharynx		+																								1
Palate, squamous cell papilloma		X																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, metastatic, skin																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma NOS																	X									1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	43
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	47
Pars distalis, adenoma		X		X	X	X	X			X	X		X	X		X		X				X	X		X	29
Pars distalis, adenoma, multiple	X																									2
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma						X					X												X		X	4
General Body System																										
Tissue NOS																										1
Schwannoma malignant, metastatic, uterus																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	4 1 9	4 5 2	4 7 7	4 9 4	5 0 4	5 2 3	5 7 2	6 2 1	6 2 4	6 4 9	6 5 2	6 5 9	6 5 9	6 7 0	6 8 1	6 8 4	6 8 4	6 9 9	7 1 3	7 2 1	7 2 6	7 3 0	7 3 3	7 3 3	7 3 3
Carcass ID Number	6 4 5	6 1 1	6 2 5	6 8 3	7 0 5	6 5 4	7 0 4	6 5 3	7 0 3	6 5 2	6 6 5	6 2 1	6 9 3	6 3 2	6 4 4	6 9 1	7 0 2	6 8 1	6 8 4	6 6 3	6 8 2	6 3 5	6 1 2	6 1 3	6 1 4
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Carcinoma					X																				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																		X							
Sarcoma, metastatic, skin		X																							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Polyp stromal														X	X	X						X		X	
Sarcoma			X																						
Schwannoma malignant										X															
Cervix, schwannoma malignant, metastatic, uterus										X															
Vagina										+															
Schwannoma malignant, metastatic, uterus										X															
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+				+				+	+	+	+	+		+		+	+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin		X																							
Schwannoma malignant, metastatic, uterus										X															
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma							X				X								X		X	X		X	
Fibroadenoma, multiple																							X		
Sarcoma, metastatic, skin		X																							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, sarcoma		X																							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	6 1 5	6 2 2	6 2 3	6 2 4	6 3 1	6 3 3	6 3 4	6 4 1	6 4 2	6 4 3	6 5 1	6 5 5	6 6 1	6 6 2	6 6 4	6 7 1	6 7 2	6 7 3	6 7 4	6 7 5	6 8 5	6 9 2	6 9 4	6 9 5	7 0 1	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor benign																										1
Sarcoma, metastatic, skin																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X													1
Polyp stromal	X				X				X		X	X										X		X		12
Sarcoma																										1
Schwannoma malignant																										1
Cervix, schwannoma malignant, metastatic, uterus																										1
Vagina																										1
Schwannoma malignant, metastatic, uterus																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node																	+	+								12
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skin																										1
Schwannoma malignant, metastatic, uterus																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma		X																								1
Fibroadenoma	X											X								X			X	X		11
Fibroadenoma, multiple																										1
Sarcoma, metastatic, skin																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma							X																			1
Subcutaneous tissue, fibrosarcoma			X																							1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	4 1 9	4 5 2	4 7 7	4 9 4	5 0 4	5 2 3	5 7 2	6 2 1	6 2 4	6 4 9	6 5 2	6 5 9	6 5 9	6 7 0	6 8 1	6 8 4	6 8 4	6 9 9	7 1 3	7 2 1	7 2 6	7 3 0	7 3 3	7 3 3	7 3 3
Carcass ID Number	6 4 5	6 1 1	6 2 5	6 8 3	7 0 5	6 5 4	7 0 4	6 5 3	7 0 3	6 5 2	6 6 5	6 2 1	6 9 3	6 3 2	6 4 4	6 9 1	6 0 2	6 8 1	6 8 4	6 6 3	6 8 2	6 3 5	6 1 2	6 1 3	6 1 4
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Sarcoma, metastatic, skin		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																							+		
Harderian gland								+																	
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									X
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X		X	X	X	X	X		X	X		X	X	X			
Lymphoma malignant lymphocytic				X																					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	6 1 5	6 2 2	6 2 3	6 2 4	6 3 1	6 3 3	6 3 4	6 4 1	6 4 2	6 4 3	6 5 1	6 5 5	6 6 1	6 6 2	6 6 4	6 7 1	6 7 2	6 7 3	6 7 4	6 7 5	6 8 5	6 9 2	6 9 4	6 9 5	7 0 1	Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Sarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye		+														+				+						4
Harderian gland																										1
Zymbal's gland	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	M	+	+	+	M	+	M	+	M	M	41
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X															X						X	X		16
Lymphoma malignant lymphocytic																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm

Number of Days on Study	3 1 7	4 2 8	5 0 6	5 3 2	5 3 3	5 4 0	5 8 3	5 8 7	6 1 0	6 1 7	6 2 9	6 3 9	6 5 2	6 6 0	6 6 9	7 0 4	7 4 8	7 6 6	7 6 6	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	8 0 2	7 4 2	7 4 3	7 7 2	8 2 2	7 3 5	7 5 4	7 8 5	7 8 3	7 7 4	7 9 3	8 1 5	7 8 4	7 3 3	7 9 5	7 6 2	7 4 5	7 4 4	7 9 2	7 3 1	7 3 2	7 3 4	7 4 1	7 5 1	7 5 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma		X																							
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery						+							+				+								
Fat, sarcoma						X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									X
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X		X	X	X		X			X		X	X	X	X	X				X	X
Pars distalis, adenoma, multiple																						X			
Pars distalis, carcinoma				X																					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma												X										X			
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma								X																	
Polyp stromal							X				X						X								
Vagina																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	7 5 3	7 5 5	7 6 1	7 6 3	7 6 4	7 6 5	7 7 1	7 7 3	7 7 5	7 8 1	7 8 2	7 9 1	7 9 4	8 0 1	8 0 3	8 0 4	8 0 5	8 1 1	8 1 2	8 1 3	8 1 4	8 2 1	8 2 3	8 2 4	8 2 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																										3
Fat, sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue												+														1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bilateral, pheochromocytoma benign															X											1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X									2
Parathyroid gland	+	M	+	+	+	+	+	+	M	M	M	+	M	+	+	M	M	+	+	+	+	+	M	+	+	39
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X	X	X			X		X	X		X	X	X		X		X	X		X	X		X	X	30
Pars distalis, adenoma, multiple															X											2
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma																										2
Follicular cell, adenoma																		X		X						2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma				X															X							2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Polyp stromal	X	X	X	X							X								X	X						10
Vagina						+				+																2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	3 1 7	4 2 8	5 0 6	5 3 2	5 3 3	5 4 0	5 8 3	5 8 7	6 1 0	6 1 7	6 2 9	6 3 9	6 5 2	6 6 0	6 6 9	6 7 4	6 8 8	7 0 6	7 2 6	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	8 0 2	7 4 2	7 4 3	7 7 2	8 2 2	7 3 5	7 5 4	7 8 5	7 8 3	7 7 4	7 9 3	8 1 5	7 8 4	7 3 3	7 9 5	6 4 2	4 4 5	4 4 4	9 2 2	3 1 1	3 3 2	3 3 4	4 1 1	5 1 1	5 5 2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+				+					+	+	+	+										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma			X																						
Fibroadenoma											X					X	X		X	X					
Fibroadenoma, multiple																									X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma NOS					X																				
Ependymoma benign																						X			
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, ear																X									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																+									
Pinna, fibrosarcoma																X									
Eye	+																			+	+	+			
Zymbal's gland	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X		X	X				X	X		X	X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	7 5 3	7 5 5	7 6 1	7 6 3	7 6 4	7 6 5	7 7 1	7 7 3	7 7 5	7 8 1	7 8 2	7 9 1	7 9 4	8 0 1	8 0 3	8 0 4	8 0 5	8 1 1	8 1 2	8 1 3	8 1 4	8 2 1	8 2 3	8 2 4	8 2 5	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										6
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	M	M	M	+	+	+	42
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma		X																								2
Fibroadenoma		X			X	X		X				X	X	X		X		X			X	X				16
Fibroadenoma, multiple															X										X	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma			X																							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma NOS																										1
Ependymoma benign																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, ear																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Ear																										1
Pinna, fibrosarcoma																										1
Eye										+									+							6
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear											X					X										9

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm

Number of Days on Study	0 8 5	1 7 6	2 2 6	2 9 6	4 8 4	5 0 2	5 0 2	5 5 4	5 5 5	5 6 0	5 7 2	5 9 5	6 1 8	6 3 7	6 3 9	6 4 9	6 5 8	6 5 8	6 5 8	6 6 0	6 6 3	6 6 5	6 7 4	7 0 2	7 1 9
Carcass ID Number	9 4 2	9 3 5	8 9 2	9 0 4	9 2 1	9 3 1	9 3 3	8 7 5	8 9 5	8 6 5	8 6 2	8 7 3	8 9 3	8 5 2	8 8 4	8 9 3	8 6 3	8 7 4	8 8 5	9 1 5	9 3 2	8 8 3	8 6 4	9 4 1	8 9 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	A	A	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery									+					+											
Pancreas	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx														+		+									
Palate, squamous cell papilloma														X		X									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																			X						
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																					X				
Bilateral, pheochromocytoma benign																X									
Islets, pancreatic	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X		X	X	X							X	X						X		
Pars distalis, adenoma, multiple																									
Pars distalis, carcinoma						X																			
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma																									
C-cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	M	+	+	+	+
Adenoma											X												X		
Squamous cell carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																				X				X	
Vagina																									
Fibroma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	8 5 1	8 5 3	8 5 4	8 5 5	8 6 1	8 7 1	8 7 2	8 8 1	8 8 2	8 9 4	9 0 1	9 0 2	9 0 3	9 0 5	9 1 1	9 1 2	9 1 4	9 2 2	9 2 3	9 2 4	9 2 5	9 3 4	9 4 3	9 4 4	9 4 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery		+				+				+												+				6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pharynx																										2
Palate, squamous cell papilloma																										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, metastatic, thyroid gland											X															1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																	X									2
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	47
Pars distalis, adenoma	X			X	X		X	X				X				X	X	X	X		X			X	X	20
Pars distalis, adenoma, multiple						X																				1
Pars distalis, carcinoma										X																2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, C-cell, adenoma													X													1
C-cell, adenoma							X														X					2
C-cell, carcinoma											X				X							X				3
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma																										2
Squamous cell carcinoma										X																1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal							X		X		X			X		X		X		X			X			10
Vagina																			+					+	+	3
Fibroma																			X							1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	0 8 5	1 7 6	2 2 6	2 9 6	4 8 4	5 0 2	5 0 2	5 5 4	5 5 5	5 6 0	5 7 2	5 9 5	6 1 8	6 3 7	6 3 9	6 4 9	6 5 8	6 5 8	6 5 8	6 6 0	6 6 3	6 6 5	7 0 4	7 1 2	7 1 9
Carcass ID Number	9 4 2	9 3 5	8 9 2	9 0 4	9 2 1	9 3 1	9 3 3	8 7 5	8 9 5	6 6 5	8 6 2	8 7 3	9 1 3	8 5 2	8 8 4	8 9 3	6 6 3	7 7 4	8 8 5	9 1 5	9 3 2	8 8 3	8 6 4	9 4 1	8 9 1
Hematopoietic System																									
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+	+						+					+		+	+				+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Thymus	A	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma						X																			
Adenocarcinoma																									
Adenoma													X												
Fibroadenoma															X				X		X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma												X													
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																							+		
Back, sarcoma																							X		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Special Senses System																									
Zymbal's gland	M	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma														X											
Urethra																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X		X			X			X		X		X	X	X			X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	8 5 1	8 5 3	8 5 4	8 5 5	8 6 1	8 7 1	8 7 2	8 8 1	8 8 2	8 8 4	8 9 1	9 0 2	9 0 3	9 0 5	9 1 1	9 1 2	9 1 4	9 2 2	9 2 3	9 2 4	9 2 5	9 3 4	9 4 3	9 4 4	9 4 5	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node		+				+	+				+	+		+	+											14
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland											X															1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	45
Thymoma benign				X																						1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoacanthoma																										1
Adenocarcinoma																X										1
Adenoma																										1
Fibroadenoma		X				X	X	X				X	X										X			10
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Back, sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pituitary gland										X																1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X						X										2
Carcinoma, metastatic, thyroid gland											X											X				2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland											X											X				2
Special Senses System																										
Zymbal's gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Urethra																		+								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X			X				X	X	X								X							16

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rates[b]	2.9%	0.0%	3.2%	9.9%
Terminal rates[c]	1/35 (3%)	0/29 (0%)	1/31 (3%)	1/25 (4%)
First incidence (days)	729 (T)	–[e]	729 (T)	649
Life table tests[d]	P=0.047	P=0.538N	P=0.735	P=0.208
Logistic regression tests[d]	P=0.063	P=0.538N	P=0.735	P=0.269
Cochran-Armitage test[d]	P=0.082			
Fisher exact test[d]		P=0.500N	P=0.753N	P=0.309
Mammary Gland: Carcinoma				
Overall rates	3/50 (6%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rates	7.9%	3.4%	5.2%	4.0%
Terminal rates	2/35 (6%)	1/29 (3%)	1/31 (3%)	1/25 (4%)
First incidence (days)	659	729 (T)	506	729 (T)
Life table tests	P=0.440N	P=0.367N	P=0.542N	P=0.431N
Logistic regression tests	P=0.369N	P=0.320N	P=0.496N	P=0.370N
Cochran-Armitage test	P=0.347N			
Fisher exact test		P=0.309N	P=0.500N	P=0.309N
Mammary Gland: Adenoma or Carcinoma				
Overall rates	3/50 (6%)	1/50 (2%)	2/50 (4%)	2/50 (4%)
Adjusted rates	7.9%	3.4%	5.2%	6.5%
Terminal rates	2/35 (6%)	1/29 (3%)	1/31 (3%)	1/25 (4%)
First incidence (days)	659	729 (T)	506	618
Life table tests	P=0.567	P=0.367N	P=0.542N	P=0.632N
Logistic regression tests	P=0.607N	P=0.320N	P=0.496N	P=0.545N
Cochran-Armitage test	P=0.591N			
Fisher exact test		P=0.309N	P=0.500N	P=0.500N
Mammary Gland: Fibroadenoma				
Overall rates	19/50 (38%)	12/50 (24%)	19/50 (38%)	10/50 (20%)
Adjusted rates	49.7%	35.4%	54.0%	34.3%
Terminal rates	16/35 (46%)	8/29 (28%)	15/31 (48%)	7/25 (28%)
First incidence (days)	666	572	629	639
Life table tests	P=0.283N	P=0.232N	P=0.402	P=0.233N
Logistic regression tests	P=0.181N	P=0.122N	P=0.462	P=0.116N
Cochran-Armitage test	P=0.069N			
Fisher exact test		P=0.097N	P=0.582N	P=0.038N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rates	19/50 (38%)	12/50 (24%)	19/50 (38%)	11/50 (22%)
Adjusted rates	49.7%	35.4%	54.0%	36.1%
Terminal rates	16/35 (46%)	8/29 (28%)	15/31 (48%)	7/25 (28%)
First incidence (days)	666	572	629	618
Life table tests	P=0.384N	P=0.232N	P=0.402	P=0.316N
Logistic regression tests	P=0.257N	P=0.122N	P=0.462	P=0.159N
Cochran-Armitage test	P=0.111N			
Fisher exact test		P=0.097N	P=0.582N	P=0.063N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rates	22/50 (44%)	13/50 (26%)	20/50 (40%)	12/50 (24%)
Adjusted rates	56.0%	38.5%	55.0%	39.6%
Terminal rates	18/35 (51%)	9/29 (31%)	15/31 (48%)	8/25 (32%)
First incidence (days)	659	572	506	618
Life table tests	P=0.316N	P=0.147N	P=0.548	P=0.228N
Logistic regression tests	P=0.178N	P=0.060N	P=0.508N	P=0.089N
Cochran-Armitage test	P=0.072N			
Fisher exact test		P=0.046N	P=0.420N	P=0.028N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	28/50 (56%)	31/47 (66%)	32/49 (65%)	21/47 (45%)
Adjusted rates	66.3%	78.1%	79.6%	67.6%
Terminal rates	21/35 (60%)	18/26 (69%)	22/30 (73%)	14/23 (61%)
First incidence (days)	499	419	533	484
Life table tests	P=0.413N	P=0.075	P=0.108	P=0.449
Logistic regression tests	P=0.098N	P=0.193	P=0.163	P=0.318N
Cochran-Armitage test	P=0.046N			
Fisher exact test		P=0.213	P=0.229	P=0.181N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rates	30/50 (60%)	31/47 (66%)	33/49 (67%)	23/47 (49%)
Adjusted rates	67.9%	78.1%	80.0%	71.8%
Terminal rates	21/35 (60%)	18/26 (69%)	22/30 (73%)	15/23 (65%)
First incidence (days)	499	419	532	484
Life table tests	P=0.502N	P=0.141	P=0.153	P=0.421
Logistic regression tests	P=0.127N	P=0.330	P=0.243	P=0.295N
Cochran-Armitage test	P=0.069N			
Fisher exact test		P=0.346	P=0.291	P=0.187N
Thyroid Gland (C-cell): Adenoma				
Overall rates	3/50 (6%)	4/50 (8%)	2/49 (4%)	3/49 (6%)
Adjusted rates	8.6%	13.8%	5.8%	12.0%
Terminal rates	3/35 (9%)	4/29 (14%)	1/30 (3%)	3/25 (12%)
First incidence (days)	729 (T)	729 (T)	639	729 (T)
Life table tests	P=0.527	P=0.397	P=0.567N	P=0.500
Logistic regression tests	P=0.546	P=0.397	P=0.534N	P=0.500
Cochran-Armitage test	P=0.557N			
Fisher exact test		P=0.500	P=0.510N	P=0.651
Thyroid Gland (C-cell): Carcinoma				
Overall rates	1/50 (2%)	0/50 (0%)	0/49 (0%)	3/49 (6%)
Adjusted rates	2.9%	0.0%	0.0%	12.0%
Terminal rates	1/35 (3%)	0/29 (0%)	0/30 (0%)	3/25 (12%)
First incidence (days)	729 (T)	–	–	729 (T)
Life table tests	P=0.028	P=0.538N	P=0.531N	P=0.193
Logistic regression tests	P=0.028	P=0.538N	P=0.531N	P=0.193
Cochran-Armitage test	P=0.051			
Fisher exact test		P=0.500N	P=0.505N	P=0.301

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rates	4/50 (8%)	4/50 (8%)	2/49 (4%)	6/49 (12%)
Adjusted rates	11.4%	13.8%	5.8%	24.0%
Terminal rates	4/35 (11%)	4/29 (14%)	1/30 (3%)	6/25 (24%)
First incidence (days)	729 (T)	729 (T)	639	729 (T)
Life table tests	P=0.117	P=0.538	P=0.408N	P=0.176
Logistic regression tests	P=0.125	P=0.538	P=0.375N	P=0.176
Cochran-Armitage test	P=0.231			
Fisher exact test		P=0.643N	P=0.349N	P=0.357
Uterus: Stromal Polyp				
Overall rates	1/50 (2%)	12/50 (24%)	10/50 (20%)	10/50 (20%)
Adjusted rates	2.9%	36.6%	28.4%	36.6%
Terminal rates	1/35 (3%)	9/29 (31%)	7/31 (23%)	8/25 (32%)
First incidence (days)	729 (T)	670	583	660
Life table tests	P=0.034	P<0.001	P=0.004	P<0.001
Logistic regression tests	P=0.053	P<0.001	P=0.004	P=0.001
Cochran-Armitage test	P=0.131			
Fisher exact test		P<0.001	P=0.004	P=0.004
All Organs: Mononuclear Cell Leukemia				
Overall rates	11/50 (22%)	16/50 (32%)	9/50 (18%)	16/50 (32%)
Adjusted rates	25.6%	39.2%	24.1%	45.3%
Terminal rates	5/35 (14%)	5/29 (17%)	4/31 (13%)	7/25 (28%)
First incidence (days)	428	621	617	554
Life table tests	P=0.080	P=0.135	P=0.503N	P=0.058
Logistic regression tests	P=0.198	P=0.187	P=0.393N	P=0.164
Cochran-Armitage test	P=0.235			
Fisher exact test		P=0.184	P=0.402N	P=0.184
All Organs: Benign Neoplasms				
Overall rates	37/50 (74%)	39/50 (78%)	39/50 (78%)	38/50 (76%)
Adjusted rates	85.9%	86.5%	92.8%	92.4%
Terminal rates	29/35 (83%)	23/29 (79%)	28/31 (90%)	22/25 (88%)
First incidence (days)	499	419	533	484
Life table tests	P=0.043	P=0.126	P=0.156	P=0.024
Logistic regression tests	P=0.218	P=0.378	P=0.284	P=0.187
Cochran-Armitage test	P=0.551			
Fisher exact test		P=0.408	P=0.408	P=0.500
All Organs: Malignant Neoplasms				
Overall rates	17/50 (34%)	22/50 (44%)	17/50 (34%)	22/50 (44%)
Adjusted rates	37.6%	47.8%	38.8%	58.2%
Terminal rates	8/35 (23%)	6/29 (21%)	6/31 (19%)	10/25 (40%)
First incidence (days)	428	452	428	502
Life table tests	P=0.092	P=0.157	P=0.473	P=0.054
Logistic regression tests	P=0.347	P=0.229	P=0.541N	P=0.186
Cochran-Armitage test	P=0.270			
Fisher exact test		P=0.206	P=0.583N	P=0.206

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rates	45/50 (90%)	46/50 (92%)	46/50 (92%)	45/50 (90%)
Adjusted rates	91.8%	92.0%	95.8%	97.8%
Terminal rates	31/35 (89%)	25/29 (86%)	29/31 (94%)	24/25 (96%)
First incidence (days)	428	419	428	484
Life table tests	P=0.050	P=0.166	P=0.224	P=0.031
Logistic regression tests	P=0.330	P=0.498	P=0.486	P=0.279
Cochran-Armitage test	P=0.513N			
Fisher exact test		P=0.500	P=0.500	P=0.630N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE B4a
Historical Incidence of Uterine Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Stromal Polyp	Stromal Sarcoma	Stromal Polyp or Sarcoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	1/50	0/50	1/50
dl-Amphetamine Sulfate	10/50	1/50	11/50
Overall Historical Incidence			
Total	205/1,251 (16.4%)	9/1,251 (0.7%)	213/1,251 (17.0%)
Standard deviation	6.6%	1.5%	6.9%
Range	2%-30%	0%-6%	2%-30%

[a] Data as of 20 August 1992

TABLE B4b
Historical Incidence of Forestomach Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	0/50	0/50	0/50
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	2/1,251 (0.2%)	0/1,251 (0.0%)	2/1,251 (0.2%)
Standard deviation	0.6%		0.6%
Range	0%-2%		0%-2%

[a] Data as of 20 August 1992

TABLE B4c
Historical Incidence of Oral Epithelium Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	1/50	0/50	1/50
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	8/1,251 (0.6%)	4/1,251 (0.3%)	12/1,251 (1.0%)
Standard deviation	1.1%	0.8%	1.4%
Range	0%-4%	0%-2%	0%-6%

[a] Data as of 20 August 1992

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	9	9	10	10
Early deaths				
Moribund	12	15	13	16
Natural deaths	4	7	6	9
Survivors				
Died last week of study		1		
Terminal sacrifice	35	28	31	25
Animals examined microscopically	59	59	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(9)	(9)	(10)	(10)
Angiectasis			1 (10%)	
Basophilic focus		3 (33%)	1 (10%)	
Clear cell focus	1 (11%)			2 (20%)
Developmental malformation	1 (11%)			
Fatty change				1 (10%)
Hepatodiaphragmatic nodule	1 (11%)			1 (10%)
Hyperplasia			1 (10%)	
Inflammation, chronic	2 (22%)	2 (22%)	3 (30%)	5 (50%)
Mesentery	(9)	(9)	(10)	(10)
Fat, necrosis	1 (11%)	1 (11%)	1 (10%)	2 (20%)
Pancreas	(9)	(9)	(10)	(10)
Atrophy, acinar cell	1 (11%)	1 (11%)	1 (10%)	2 (20%)
Cardiovascular System				
None				
Endocrine System				
Pituitary gland	(9)	(8)	(10)	(10)
Pars distalis, angiectasis			1 (10%)	2 (20%)
Pars distalis, cyst	4 (44%)	4 (50%)		4 (40%)
Pars distalis, hyperplasia	3 (33%)		5 (50%)	5 (50%)
Thyroid gland	(9)	(8)	(10)	(10)
C-cell, hyperplasia	1 (11%)	1 (13%)		3 (30%)
General Body System				
None				
Genital System				
Clitoral gland	(9)	(9)	(10)	(10)
Duct, dilatation			2 (20%)	
Inflammation, chronic	1 (11%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Genital System (continued)				
Ovary	(9)	(9)	(10)	(10)
Cyst		1 (11%)		1 (10%)
Cyst, bilateral				1 (10%)
Uterus	(9)	(9)	(10)	(10)
Cyst		3 (33%)		
Dilatation	2 (22%)	1 (11%)	2 (20%)	4 (40%)
Hematopoietic System				
Lymph node, mandibular	(8)	(9)	(10)	(10)
Congestion	4 (50%)	2 (22%)	3 (30%)	2 (20%)
Thymus	(9)	(9)	(10)	(10)
Hemorrhage			1 (10%)	
Integumentary System				
Mammary gland	(9)	(9)	(9)	(10)
Dilatation	1 (11%)			
Musculoskeletal System				
None				
Nervous System				
Brain	(9)	(9)	(10)	(10)
Inflammation, chronic				1 (10%)
Respiratory System				
Lung	(9)	(9)	(10)	(10)
Congestion	1 (11%)			1 (10%)
Hemmorrhage			1 (10%)	
Infiltration cellular, histiocyte	3 (33%)		5 (50%)	1 (10%)
Interstitum, inflammmation	1 (11%)	2 (22%)	1 (10%)	1 (10%)
Nose	(9)	(9)	(10)	(10)
Fungus			1 (10%)	
Inflammation, chronic, active		1 (11%)	1 (10%)	
Special Senses System				
None				
Urinary System				
Kidney	(9)	(9)	(10)	(10)
Mineralization	8 (89%)	7 (78%)	9 (90%)	10 (100%)
Nephropathy	5 (56%)	3 (33%)	6 (60%)	8 (80%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study				
Alimentary System				
Intestine small, jejunum	(48)	(45)	(49)	(47)
Mineralization, focal	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal	3 (6%)	1 (2%)		
Basophilic focus	38 (76%)	28 (56%)	41 (82%)	30 (60%)
Clear cell focus	11 (22%)	4 (8%)	16 (32%)	12 (24%)
Congestion	2 (4%)	1 (2%)	3 (6%)	
Cyst			1 (2%)	
Degeneration, cystic, focal	2 (4%)			1 (2%)
Developmental malformation	1 (2%)	5 (10%)	1 (2%)	
Eosinophilic focus	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Fatty change, diffuse	4 (8%)	5 (10%)	2 (4%)	1 (2%)
Fatty change, focal	14 (28%)	11 (22%)	9 (18%)	18 (36%)
Hematopoietic cell proliferation	3 (6%)	1 (2%)		
Hemorrhage, focal		1 (2%)		
Hepatodiaphragmatic nodule	4 (8%)	4 (8%)	2 (4%)	1 (2%)
Hyperplasia, focal	4 (8%)	1 (2%)	1 (2%)	
Inflammation, chronic, focal	11 (22%)	9 (18%)	8 (16%)	10 (20%)
Inflammation, focal, granulomatous	1 (2%)	2 (4%)	1 (2%)	
Mixed cell focus	2 (4%)	2 (4%)		4 (8%)
Necrosis, focal		2 (4%)	1 (2%)	3 (6%)
Bile duct, dilatation, focal			1 (2%)	
Bile duct, hyperplasia, focal	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Centrilobular, necrosis				2 (4%)
Vein, dilatation		1 (2%)	1 (2%)	
Mesentery	(8)	(6)	(3)	(6)
Fat, necrosis, focal	8 (100%)	3 (50%)	2 (67%)	5 (83%)
Pancreas	(50)	(48)	(50)	(48)
Accessory spleen				1 (2%)
Acinus, atrophy, diffuse	1 (2%)			1 (2%)
Acinus, atrophy, focal	15 (30%)	9 (19%)	9 (18%)	13 (27%)
Acinus, ectopic liver, focal				1 (2%)
Artery, inflammation, focal				1 (2%)
Duct, hyperplasia, focal				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Stomach, forestomach	(50)	(49)	(50)	(49)
Acanthosis, focal	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Hyperplasia, basal cell, focal	1 (2%)	1 (2%)	5 (10%)	11 (22%)
Inflammation, acute, focal	2 (4%)			
Inflammation, chronic, focal	1 (2%)	1 (2%)		1 (2%)
Mineralization, focal			1 (2%)	
Ulcer	1 (2%)	1 (2%)		1 (2%)
Ulcer, focal	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Stomach, glandular	(50)	(48)	(50)	(48)
Erosion, focal	1 (2%)		1 (2%)	
Inflammation, chronic active, focal	1 (2%)			
Ulcer, focal			1 (2%)	
Tongue			(1)	
Acanthosis, focal			1 (100%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	22 (44%)	20 (40%)	21 (42%)	14 (28%)
Atrium, thrombosis		2 (4%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Angiectasis, focal	2 (4%)	5 (10%)	13 (26%)	7 (14%)
Congestion				2 (4%)
Cyst		1 (2%)	1 (2%)	
Degeneration, cystic, focal		2 (4%)	1 (2%)	
Hematopoietic cell proliferation, focal			1 (2%)	
Hemorrhage			1 (2%)	
Hyperplasia, focal	4 (8%)	3 (6%)	7 (14%)	6 (12%)
Hypertrophy, focal	2 (4%)	3 (6%)	2 (4%)	4 (8%)
Necrosis, focal				2 (4%)
Vacuolization cytoplasmic, diffuse		2 (4%)		
Vacuolization cytoplasmic, focal	18 (36%)	21 (42%)	16 (32%)	18 (37%)
Adrenal medulla	(49)	(50)	(47)	(49)
Degeneration		1 (2%)		
Hyperplasia	7 (14%)	5 (10%)	6 (13%)	3 (6%)
Bilateral, hyperplasia		2 (4%)	1 (2%)	1 (2%)
Islets, pancreatic	(50)	(48)	(50)	(48)
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(45)	(43)	(39)	(44)
Cyst				1 (2%)
Pituitary gland	(50)	(47)	(49)	(47)
Hemorrhage, focal			1 (2%)	
Pars distalis, angiectasis		2 (4%)		1 (2%)
Pars distalis, angiectasis, focal	1 (2%)			
Pars distalis, cyst	10 (20%)	10 (21%)	7 (14%)	11 (23%)
Pars distalis, hyperplasia, focal	13 (26%)	9 (19%)	9 (18%)	14 (30%)
Thyroid gland	(50)	(50)	(49)	(49)
Inflammation	1 (2%)	1 (2%)		
C-cell, hyperplasia, diffuse	1 (2%)			
C-cell, hyperplasia, focal	10 (20%)	4 (8%)	6 (12%)	11 (22%)
Follicle, dilatation, focal		1 (2%)		
General Body System				
None				
Genital System				
Clitoral gland	(47)	(48)	(49)	(47)
Hyperplasia			3 (6%)	3 (6%)
Inflammation, chronic	2 (4%)			1 (2%)
Inflammation, suppurative	3 (6%)		1 (2%)	2 (4%)
Bilateral, duct, dilatation			1 (2%)	
Duct, dilatation	8 (17%)	12 (25%)	15 (31%)	9 (19%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Genital System (continued)				
Ovary	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Cyst	3 (6%)	9 (18%)	5 (10%)	4 (8%)
Bilateral, cyst				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Cyst	5 (10%)	7 (14%)	8 (16%)	6 (12%)
Cyst, multiple	2 (4%)	3 (6%)		2 (4%)
Dilatation	3 (6%)	5 (10%)	6 (12%)	
Hypoplasia	1 (2%)			
Inflammation, acute			1 (2%)	
Vagina	(1)	(1)	(2)	(3)
Cyst			2 (100%)	
Hyperplasia, focal, squamous			1 (50%)	
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(49)
Atrophy	7 (14%)	4 (8%)	10 (20%)	8 (16%)
Hyperplasia		1 (2%)		
Lymph node	(7)	(12)	(6)	(14)
Axillary, hyperplasia, lymphoid				1 (7%)
Axillary, hyperplasia, plasma cell			1 (17%)	
Iliac, hyperplasia, plasma cell		1 (8%)		
Mediastinal, hemorrhage	1 (14%)	2 (17%)	3 (50%)	4 (29%)
Mediastinal, infiltration cellular, histiocyte		1 (8%)		
Pancreatic, infiltration cellular, histiocyte				1 (7%)
Renal, hemorrhage	2 (29%)	1 (8%)	1 (17%)	2 (14%)
Lymph node, mandibular	(49)	(50)	(50)	(50)
Cyst		3 (6%)		
Hemorrhage	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Hyperplasia, plasma cell	3 (6%)	1 (2%)		3 (6%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemorrhage	6 (12%)	4 (8%)	3 (6%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)	
Hyperplasia, plasma cell		1 (2%)		
Spleen	(50)	(49)	(50)	(48)
Hematopoietic cell proliferation	1 (2%)		2 (4%)	1 (2%)
Inflammation, chronic, focal		1 (2%)		
Thymus	(48)	(49)	(42)	(45)
Congestion			1 (2%)	
Cyst, multiple		1 (2%)		
Hemorrhage	1 (2%)			2 (4%)
Necrosis				1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Degeneration, focal	1 (2%)			
Galactocele	13 (26%)	14 (28%)	10 (20%)	17 (34%)
Hyperplasia, focal	1 (2%)	1 (2%)		4 (8%)
Inflammation, acute, focal	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic, focal	1 (2%)	2 (4%)		1 (2%)
Duct, dilatation	2 (4%)		1 (2%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)			1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	5 (10%)	2 (4%)	5 (10%)	1 (2%)
Inflammation, acute	1 (2%)			
Femur, osteoporosis	1 (2%)			
Skeletal muscle	(1)			(1)
Diaphragm, ectopic tissue	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage, focal		1 (2%)	2 (4%)	
Hydrocephalus	1 (2%)	6 (12%)	3 (6%)	5 (10%)
Infiltration cellular, focal, lymphocyte				1 (2%)
Inflammation, focal		1 (2%)		
Meninges, angiectasis, focal		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)		2 (4%)	5 (10%)
Hemorrhage, focal	1 (2%)	3 (6%)	2 (4%)	
Infiltration cellular, focal, lymphocyte		1 (2%)	2 (4%)	
Infiltration cellular, focal, histiocyte	10 (20%)	7 (14%)	7 (14%)	11 (22%)
Inflammation, focal	9 (18%)	6 (12%)	11 (22%)	8 (16%)
Alveolar epithelium, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Arteriole, mineralization, focal				1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body		1 (2%)		1 (2%)
Fungus	1 (2%)	1 (2%)	6 (12%)	8 (16%)
Hemorrhage	1 (2%)		2 (4%)	
Inflammation, acute	2 (4%)	3 (6%)	2 (4%)	3 (6%)
Inflammation, chronic active	6 (12%)	6 (12%)	9 (18%)	9 (18%)
Inflammation, subacute	2 (4%)	5 (10%)	3 (6%)	4 (8%)
Goblet cell, hyperplasia			1 (2%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Special Senses System				
Eye	(2)	(4)	(6)	
Hemorrhage		1 (25%)		
Inflammation, acute			1 (17%)	
Phthisis bulbi	1 (50%)			
Bilateral, lens, cataract		2 (50%)	3 (50%)	
Bilateral, retina, atrophy		2 (50%)	3 (50%)	
Lens, cataract		1 (25%)	1 (17%)	
Retina, atrophy	1 (50%)	1 (25%)	1 (17%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Cyst		1 (2%)	1 (2%)	
Cyst, multiple			1 (2%)	
Hemorrhage, focal	1 (2%)			
Infiltration cellular, focal, lymphocyte	1 (2%)			
Mineralization, focal	32 (64%)	25 (50%)	28 (56%)	34 (68%)
Nephropathy	39 (78%)	38 (76%)	41 (82%)	40 (80%)
Pigmentation, focal	34 (68%)	24 (48%)	28 (56%)	42 (84%)
Bilateral, hydronephrosis		1 (2%)		
Urethra				(1)
Cyst				1 (100%)
Urinary bladder	(48)	(48)	(50)	(49)
Hemorrhage				1 (2%)
Mucosa, hyperplasia		1 (2%)		

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF C.I. DIRECT BLUE 218

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	9	10	10	10
Early deaths				
Accidental deaths	1			
Moribund	2	2	3	1
Natural deaths	3	2	5	4
Survivors				
Terminal sacrifice	44	46	42	45
Missing	1			
Animals examined microscopically	59	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Intestine small, jejuunum	(9)			(10)
Adenocarcinoma				1 (10%)
Liver	(9)	(10)	(10)	(10)
Hepatocellular adenoma				1 (10%)
Hepatocellular carcinoma	1 (11%)			
Cardiovascular System				
None				
Endocrine System				
Thyroid	(9)			(10)
Follicular cell, adenocarcinoma	1 (11%)			
General Body System				
None				
Genital System				
None				
Hematopoietic System				
None				
Integumentary System				
None				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(9)	(3)	(2)	(10)
Alveolar/bronchiolar adenoma	2 (22%)	2 (67%)	1 (50%)	
Urinary System				
None				
Special Senses System				
None				
Systemic Lesions				
None				
2-Year Study				
Alimentary System				
Gallbladder	(45)	(47)	(46)	(47)
Intestine large, colon	(48)	(50)	(50)	(49)
Intestine large, cecum	(48)	(49)	(48)	(48)
Intestine small, duodenum	(48)	(48)	(47)	(49)
Adenoma		1 (2%)		
Intestine small, jejunum	(49)	(49)	(49)	(49)
Carcinoma	1 (2%)			3 (6%)
Intestine small, ileum	(49)	(49)	(47)	(48)
Liver	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Hemangiosarcoma			1 (2%)	1 (2%)
Hemangiosarcoma, metastatic, spleen		1 (2%)		1 (2%)
Hepatoblastoma				1 (2%)
Hepatocellular carcinoma	7 (14%)	3 (6%)	8 (16%)	17 (34%)
Hepatocellular adenoma	12 (24%)	9 (18%)	7 (14%)	9 (18%)
Hepatocellular adenoma, multiple	4 (8%)	10 (20%)	10 (20%)	31 (62%)
Histiocytic sarcoma	1 (2%)			1 (2%)
Mast cell tumor malignant, metastatic, spleen	1 (2%)			
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Mesentery	(3)	(1)		
Sarcoma, metastatic, uncertain primary site		1 (100%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Pancreas	(49)	(50)	(50)	(50)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)		1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Sarcoma			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		3 (6%)	1 (2%)	
Capsule, adenoma		1 (2%)		
Pituitary gland	(49)	(48)	(48)	(48)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	2 (4%)	2 (4%)		2 (4%)
General Body System				
Tissue NOS	(1)	(2)	(1)	
Sarcoma, metastatic, uncertain primary site		1 (50%)		
Abdominal, hemangiosarcoma, metastatic, spleen		1 (50%)		
Genital System				
Ductus deferens				(1)
Epididymis	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Sarcoma	1 (2%)	1 (2%)		
Preputial gland	(29)	(23)	(23)	(21)
Prostate	(50)	(49)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Interstitial cell, adenoma		1 (2%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Mast cell tumor malignant, metastatic, spleen	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node	(4)	(1)	(2)	(3)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)	
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)	
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)	
Lymph node, mandibular	(46)	(49)	(49)	(48)
Lymph node, mesenteric	(48)	(49)	(50)	(50)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Spleen	(49)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma		1 (2%)		1 (2%)
Histiocytic sarcoma				1 (2%)
Mast cell tumor malignant	1 (2%)			
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Thymus	(49)	(45)	(46)	(42)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibroma		1 (2%)		
Subcutaneous tissue, hemangioma				1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)			
Musculoskeletal System				
Skeletal muscle		(1)		
Sarcoma		1 (100%)		
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	12 (24%)	8 (16%)	7 (14%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)		3 (6%)	3 (6%)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Nose	(50)	(50)	(50)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Special Senses System				
Ear	(2)			
Pinna, sarcoma	1 (50%)			
Harderian gland	(3)	(4)	(4)	
Adenoma	3 (100%)	3 (75%)	4 (100%)	
Bilateral, adenoma		1 (25%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma		2 (4%)	1 (2%)	1 (2%)
Renal tubule, carcinoma		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			1 (2%)
Lymphoma malignant lymphocytic	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Lymphoma malignant mixed		3 (6%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]	31	37	28	46
Total primary neoplasms	51	59	46	78
Total animals with benign neoplasms	25	31	21	42
Total benign neoplasms	35	45	31	51
Total animals with malignant neoplasms	14	14	12	24
Total malignant neoplasms	16	14	15	27
Total animals with metastatic neoplasms	2	3	4	4
Total metastatic neoplasm	3	10	6	4
Total animals with malignant neoplasms uncertain primary site		1		

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm

Number of Days on Study	0 2 4	3 8 8	5 1 9	6 1 3	6 6 7	6 7 6	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9
Carcass ID Number	0 0 9 1	0 4 2 1	0 2 3 1	0 2 2 1	0 1 9 1	0 4 1 1	0 0 1 1	0 0 2 1	0 0 3 1	0 0 4 1	0 0 5 1	0 0 6 1	0 0 7 1	0 0 8 1	0 1 0 1	0 1 1 1	0 1 2 1	0 1 3 1	0 1 4 1	0 1 5 1	0 1 6 1	0 1 7 1	0 1 8 1	0 2 0 1	0 2 1 1
Alimentary System																									
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	M	M
Intestine large, colon	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma										X				X											
Hepatocellular adenoma			X	X								X		X								X			
Hepatocellular adenoma, multiple																		X		X				X	
Histiocytic sarcoma															X										
Mast cell tumor malignant, metastatic, spleen																									
Mesentery							+												+				+		
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	M	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma							X																		
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Sarcoma																									
Preputial gland					+		+	+	+	+	+			+	+	+		+	+	+	+			+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	
Carcass ID Number	0 2 4 1	0 2 5 1	0 2 6 1	0 2 7 1	0 2 8 1	0 2 9 1	0 3 0 1	0 3 1 1	0 3 2 1	0 3 3 1	0 3 4 1	0 3 5 1	0 3 6 1	0 3 7 1	0 3 8 1	0 3 9 1	0 4 0 1	0 4 3 1	0 4 4 1	0 4 5 1	0 4 6 1	0 4 7 1	0 4 8 1	0 4 9 1	0 5 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma														X												1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma	X	X																X	X				X			7
Hepatocellular adenoma		X	X									X		X	X		X			X						12
Hepatocellular adenoma, multiple							X																			4
Histiocytic sarcoma																										1
Mast cell tumor malignant, metastatic, spleen														X												1
Mesentery																										3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma										X																1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	M	+	41
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma										X																2
General Body System																										
Tissue NOS															+											1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Sarcoma			X																							1
Preputial gland	+		+	+		+		+	+		+		+	+		+			+	+			+	+		29
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	0 2 4	3 8 8	5 1 9	6 1 3	6 6 7	6 7 6	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9
Carcass ID Number	0 0 9 1	0 4 2 1	0 2 3 1	0 2 2 1	0 1 9 1	0 4 1 1	0 0 1 1	0 0 2 1	0 0 3 1	0 0 4 1	0 0 5 1	0 0 6 1	0 0 7 1	0 0 8 1	0 1 0 1	0 1 1 1	0 1 2 1	0 1 3 1	0 1 4 1	0 1 5 1	0 1 6 1	0 1 7 1	0 1 8 1	0 2 0 1	0 2 1 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Mast cell tumor malignant, metastatic, spleen																									
Lymph node						+												+							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	I	I	I	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, lipoma									X																
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X		X		X		X		X						X				X			
Alveolar/bronchiolar adenoma, multiple									X																
Alveolar/bronchiolar carcinoma															X										
Hepatocellular carcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																								+	
Pinna, sarcoma																								X	
Eye				+																					
Harderian gland				+																+					
Adenoma				X																X					
Zymbal's gland			+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Lymphoma malignant lymphocytic						X																			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	
Carcass ID Number	0 2 4 1	0 2 5 1	0 2 6 1	0 2 7 1	0 2 8 1	0 2 9 1	0 3 0 1	0 3 1 1	0 3 2 1	0 3 3 1	0 3 4 1	0 3 5 1	0 3 6 1	0 3 7 1	0 3 8 1	0 3 9 1	0 4 0 1	0 4 3 1	0 4 4 1	0 4 5 1	0 4 6 1	0 4 7 1	0 4 8 1	0 4 9 1	0 5 0 1	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Mast cell tumor malignant, metastatic, spleen														X												1
Lymph node								+					+													4
Lymph node, mandibular	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mast cell tumor malignant														X												1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, lipoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X	X	X			X	X														12
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma																										1
Hepatocellular carcinoma, metastatic, liver	X																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear													+													2
Pinna, sarcoma																										1
Eye																										1
Harderian gland																	+									3
Adenoma																	X									3
Zymbal's gland	M	M	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	39
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant lymphocytic													X													2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm

Number of Days on Study	4 5 5	6 5 3	6 5 9	6 8 9	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8
Carcass ID Number	0 7 8 1	1 0 2 1	0 8 4 1	0 7 1 1	0 6 1 1	0 6 2 1	0 6 3 1	0 6 4 1	0 6 5 1	0 6 6 1	0 6 7 1	0 6 8 1	0 6 9 1	0 7 0 1	0 7 2 1	0 7 3 1	0 7 4 1	0 7 5 1	0 7 6 1	0 7 7 1	0 7 9 1	0 8 0 1	0 8 1 1	0 8 2 1	0 8 3 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen			X																						
Hepatocellular carcinoma					X												X					X			
Hepatocellular adenoma												X	X		X		X				X	X			
Hepatocellular adenoma, multiple				X						X						X								X	
Sarcoma, metastatic, uncertain primary site				X																					
Mesentery				+																					
Sarcoma, metastatic, uncertain primary site				X																					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, uncertain primary site				X																					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								X	
General Body System																									
Tissue NOS			+	+																					
Sarcoma, metastatic, uncertain primary site				X																					
Abdominal, hemangiosarcoma, metastatic, spleen			X																						

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	
Carcass ID Number	0 8 5 1	0 8 6 1	0 8 7 1	0 8 8 1	0 8 9 1	0 9 0 1	0 9 1 1	0 9 2 1	0 9 3 1	0 9 4 1	0 9 5 1	0 9 6 1	0 9 7 1	0 9 8 1	0 9 9 1	1 0 0 1	1 0 1 1	1 0 3 1	1 0 4 1	1 0 5 1	1 0 6 1	1 0 7 1	1 0 8 1	1 0 9 1	1 1 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																								X		1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, spleen																										1
Hepatocellular carcinoma																										3
Hepatocellular adenoma								X					X	X												9
Hepatocellular adenoma, multiple	X		X				X				X				X						X					10
Sarcoma, metastatic, uncertain primary site																										1
Mesentery																										1
Sarcoma, metastatic, uncertain primary site																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, uncertain primary site																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma											X															1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X													X										3
Capsule, adenoma								X																		1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	M	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	43
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																					X					2
General Body System																										
Tissue NOS																										2
Sarcoma, metastatic, uncertain primary site																										1
Abdominal, hemangiosarcoma, metastatic, spleen																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	4 5 5	6 5 3	6 5 9	6 8 9	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8
Carcass ID Number	0 7 8 1	1 0 2 1	0 8 4 1	0 7 1 1	0 6 1 1	0 6 2 1	0 6 3 1	0 6 4 1	0 6 5 1	0 6 6 1	0 6 7 1	0 6 8 1	0 6 9 1	0 7 0 1	0 7 2 1	0 7 3 1	0 7 4 1	0 7 5 1	0 7 6 1	0 7 7 1	0 7 9 1	0 8 0 1	0 8 1 1	0 8 2 1	0 8 3 1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																										
Preputial gland			+				+			+		+		+			+			+	+		+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																					X					
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, uncertain primary site				X																						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma						X																				
Hemangiosarcoma			X																							
Sarcoma, metastatic, uncertain primary site				X																						
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	+	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																			X							
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+																								
Sarcoma		X																								
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X									X		X		X						X		
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma										X																
Sarcoma, metastatic, uncertain primary site				X																						
Sarcoma, metastatic, skeletal muscle		X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	
Carcass ID Number	0 8 5 1	0 8 6 1	0 8 7 1	0 8 8 1	0 8 9 1	0 9 0 1	0 9 1 1	0 9 2 1	0 9 3 1	0 9 4 1	0 9 5 1	0 9 6 1	0 9 7 1	0 9 8 1	0 9 9 1	1 0 0 1	1 0 1 1	1 0 3 1	1 0 4 1	1 0 5 1	1 0 6 1	1 0 7 1	1 0 8 1	1 0 9 1	1 1 0 1	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma									X																	1
Preputial gland		+	+	+	+	+		+	+		+				+	+		+				+			23
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, metastatic, uncertain primary site																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Hemangiosarcoma																										1
Sarcoma, metastatic, uncertain primary site																										1
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X							X	X																8
Alveolar/bronchiolar adenoma, multiple																X										1
Alveolar/bronchiolar carcinoma					X			X																		3
Sarcoma, metastatic, uncertain primary site																										1
Sarcoma, metastatic, skeletal muscle																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	4 5 5	6 5 3	6 5 9	6 8 9	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8
Carcass ID Number	0 7 8 1	1 0 2 1	0 8 4 1	0 7 1 1	0 6 1 1	0 6 2 1	0 6 3 1	0 6 4 1	0 6 5 1	0 6 6 1	0 6 7 1	0 6 8 1	0 6 9 1	0 7 0 1	0 7 2 1	0 7 3 1	0 7 4 1	0 7 5 1	0 7 6 1	0 7 7 1	0 7 9 1	0 8 0 1	0 8 1 1	0 8 2 1	0 8 3 1
Special Senses System																									
Eye										+								+							
Harderian gland										+								+		+					
Adenoma										X								X		X					
Bilateral, adenoma																									
Zymbal's gland		+	+	+	M	+	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+	M	M	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed	X					X																			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	
Carcass ID Number	0 8 5 1	0 8 6 1	0 8 7 1	0 8 8 1	0 8 9 1	0 9 0 1	0 9 1 1	0 9 2 1	0 9 3 1	0 9 4 1	0 9 5 1	0 9 6 1	0 9 7 1	0 9 8 1	0 9 9 1	1 0 0 1	1 0 1 1	1 0 3 1	1 0 4 1	1 0 5 1	1 0 6 1	1 0 7 1	1 0 8 1	1 0 9 1	1 1 0 1	Total Tissues/ Tumors
Special Senses System																										
Eye																										2
Harderian gland			+																							4
Adenoma																										3
Bilateral, adenoma			X																							1
Zymbal's gland	+	+	M	+	M	M	M	+	+	+	+	+	M	+	+	M	M	M	+	+	+	+	+	M	+	34
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma	X			X																						2
Renal tubule, carcinoma																		X								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic			X																							1
Lymphoma malignant mixed																									X	3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm

Number of Days on Study	5 1 1	6 2 2	6 4 6	6 5 0	6 5 5	6 8 6	7 0 2	7 0 4	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8
Carcass ID Number	1 3 1 1	1 4 4 1	1 2 9 1	1 4 9 1	1 6 5 1	1 5 6 1	1 4 2 1	1 3 8 1	1 2 1 1	1 2 2 1	1 2 3 1	1 2 4 1	1 2 5 1	1 2 6 1	1 2 7 1	1 2 8 1	1 3 0 1	1 3 2 1	1 3 3 1	1 3 4 1	1 3 5 1	1 3 6 1	1 3 7 1	1 3 9 1	1 4 0 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	I	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																						
Hepatocellular carcinoma		X	X			X	X																		
Hepatocellular adenoma						X				X			X						X	X					
Hepatocellular adenoma, multiple								X			X	X									X	X	X		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma			X																						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	I	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS																		+							
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland		+			+	+	+				+				+		+			+	+	+		+	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	1 4 1 1	1 4 3 1	1 4 5 1	1 4 6 1	1 4 7 1	1 4 8 1	1 5 0 1	1 5 1 1	1 5 2 1	1 5 3 1	1 5 4 1	1 5 5 1	1 5 7 1	1 5 8 1	1 5 9 1	1 6 0 1	1 6 1 1	1 6 2 1	1 6 3 1	1 6 4 1	1 6 6 1	1 6 7 1	1 6 8 1	1 6 9 1	1 7 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatocellular carcinoma				X								X	X					X								8
Hepatocellular adenoma										X		X														7
Hepatocellular adenoma, multiple														X	X				X			X				10
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M	M	+	M	+	+	+	+	42
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+			+				+		+	+	+		+	+				+		+		+		23
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma	X																									1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	5 1 1	6 2 2	6 4 6	6 5 0	6 5 5	6 8 6	7 0 2	7 0 4	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8
Carcass ID Number	1 3 1 1	1 4 4 1	1 2 9 1	1 4 9 1	1 6 5 1	1 5 6 1	1 4 2 1	1 3 8 1	1 2 1 1	1 2 2 1	1 2 3 1	1 2 4 1	1 2 5 1	1 2 6 1	1 2 7 1	1 2 8 1	1 3 0 1	1 3 2 1	1 3 3 1	1 3 4 1	1 3 5 1	1 3 6 1	1 3 7 1	1 3 9 1	1 4 0 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node								+														+			
Alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	I	M	I	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X										X					
Alveolar/bronchiolar carcinoma								X																	
Hepatocellular carcinoma, metastatic, liver		X	X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																						+			
Harderian gland						+					+											+			
Adenoma						X					X											X			
Zymbal's gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	M	+	M
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																		X							

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	1 4 1 1	1 4 3 1	1 4 5 1	1 4 6 1	1 4 7 1	1 4 8 1	1 5 0 1	1 5 1 1	1 5 2 1	1 5 3 1	1 5 4 1	1 5 5 1	1 5 7 1	1 5 8 1	1 5 9 1	1 6 0 1	1 6 1 1	1 6 2 1	1 6 3 1	1 6 4 1	1 6 6 1	1 6 7 1	1 6 8 1	1 6 9 1	1 7 0 1	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										2
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma						X																				1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X		X							X							X		X							7
Alveolar/bronchiolar carcinoma														X												2
Hepatocellular carcinoma, metastatic, liver																		X								3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																			+							2
Harderian gland																			+							4
Adenoma																			X							4
Zymbal's gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	42
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma															X											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic				X																						2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm

Number of Days on Study	5 6 8	5 7 6	6 6 0	6 7 5	7 1 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7
Carcass ID Number	2 2 9 1	1 9 8 1	2 2 6 1	2 1 3 1	2 0 2 1	1 8 1 1	1 8 2 1	1 8 3 1	1 8 4 1	1 8 5 1	1 8 6 1	1 8 7 1	1 8 8 1	1 8 9 1	1 9 0 1	1 9 1 1	1 9 2 1	1 9 3 1	1 9 4 1	1 9 5 1	1 9 6 1	1 9 7 1	1 9 9 1	2 0 0 1	2 0 1 1
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																										
Intestine small, ileum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																										
Hemangiosarcoma			X																							
Hemangiosarcoma, metastatic, spleen				X																						
Hepatoblastoma																										
Hepatocellular carcinoma	X	X				X					X	X		X			X						X	X		
Hepatocellular adenoma			X		X												X									X
Hepatocellular adenoma, multiple		X		X		X		X		X	X	X	X	X	X	X		X	X		X	X		X		
Histiocytic sarcoma																X										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																										
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																										
General Body System																										
None																										
Genital System																										
Ductus deferens																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+				+						+						+	+	+		+				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma												X														

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 0 3 1	2 0 4 1	2 0 5 1	2 0 6 1	2 0 7 1	2 0 8 1	2 0 9 1	2 1 0 1	2 1 1 1	2 1 2 1	2 1 4 1	2 1 5 1	2 1 6 1	2 1 7 1	2 1 8 1	2 1 9 1	2 2 0 1	2 2 1 1	2 2 2 1	2 2 3 1	2 2 4 1	2 2 5 1	2 2 7 1	2 2 8 1	2 3 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma			X								X													X		3
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																									X	1
Hemangiosarcoma																										1
Hemangiosarcoma, metastatic, spleen																										1
Hepatoblastoma																X										1
Hepatocellular carcinoma	X								X		X		X			X	X	X			X					17
Hepatocellular adenoma		X		X							X						X				X					9
Hepatocellular adenoma, multiple			X		X	X	X	X		X		X	X	X	X	X			X	X			X		X	31
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma											X															1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma		X			X																					2
General Body System																										
None																										
Genital System																										
Ductus deferens							+																			1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland			+	+	+	+	+	+		+		+	+			+			+		+			+		21
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	5 6 8	5 7 6	6 6 0	6 7 5	7 1 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7
Carcass ID Number	2 2 9 1	1 9 8 1	2 2 6 1	2 1 3 1	2 0 2 1	1 8 1 1	1 8 2 1	1 8 3 1	1 8 4 1	1 8 5 1	1 8 6 1	1 8 7 1	1 8 8 1	1 8 9 1	1 9 0 1	1 9 1 1	1 9 2 1	1 9 3 1	1 9 4 1	1 9 5 1	1 9 6 1	1 9 7 1	1 9 9 1	2 0 0 1	2 0 1 1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+																						
Lymph node, mandibular	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X																						
Histiocytic sarcoma																X										
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	M	+	+
Integumentary System																										
Mammary gland	M	M	M	I	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	+	M	M	+	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangioma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X													X				X			
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma																										
Hepatocellular carcinoma, metastatic, liver	X																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Zymbal's gland	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																X										
Lymphoma malignant lymphocytic																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 0 3 1	2 0 4 1	2 0 5 1	2 0 6 1	2 0 7 1	2 0 8 1	2 0 9 1	2 1 0 1	2 1 1 1	2 1 2 1	2 1 4 1	2 1 5 1	2 1 6 1	2 1 7 1	2 1 8 1	2 1 9 1	2 2 0 1	2 2 1 1	2 2 2 1	2 2 3 1	2 2 4 1	2 2 5 1	2 2 7 1	2 2 8 1	2 3 0 1	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node							+																	+		3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	M	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	42
Integumentary System																										
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangioma				X																						1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										3
Alveolar/bronchiolar adenoma, multiple													X													1
Alveolar/bronchiolar carcinoma														X												1
Hepatocellular carcinoma, metastatic, liver											X							X								3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Zymbal's gland	+	M	M	M	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	M	M	M	+	+	37
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma									X																	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant lymphocytic							X																	X		2

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Adrenal Cortex: Adenoma				
Overall rates[a]	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rates[b]	0.0%	6.5%	2.4%	0.0%
Terminal rates[c]	0/44 (0%)	3/46 (7%)	1/42 (2%)	0/45 (0%)
First incidence (days)	–[e]	737 (T)	737 (T)	–
Life table tests[d]	P=0.280N	P=0.129	P=0.491	–
Logistic regression tests[d]	P=0.280N	P=0.129	P=0.491	–
Cochran-Armitage test[d]	P=0.280N			
Fisher exact test[d]		P=0.121	P=0.500	–
Harderian Gland: Adenoma				
Overall rates	3/50 (6%)	4/50 (8%)	4/50 (8%)	0/50 (0%)
Adjusted rates	6.6%	8.7%	9.2%	0.0%
Terminal rates	2/44 (5%)	4/46 (9%)	3/42 (7%)	0/45 (0%)
First incidence (days)	613	737 (T)	686	–
Life table tests	P=0.067N	P=0.524	P=0.487	P=0.119N
Logistic regression tests	P=0.065N	P=0.504	P=0.497	P=0.127N
Cochran-Armitage test	P=0.066N			
Fisher exact test		P=0.500	P=0.500	P=0.121N
Kidney (Renal Tubule): Adenoma or Carcinoma				
Overall rates	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rates	0.0%	6.5%	2.4%	2.2%
Terminal rates	0/44 (0%)	3/46 (7%)	1/42 (2%)	1/45 (2%)
First incidence (days)	–	737 (T)	737 (T)	737 (T)
Life table tests	P=0.588N	P=0.129	P=0.491	P=0.504
Logistic regression tests	P=0.588N	P=0.129	P=0.491	P=0.504
Cochran-Armitage test	P=0.592N			
Fisher exact test		P=0.121	P=0.500	P=0.500
Liver: Hepatocellular Adenoma				
Overall rates	16/50 (32%)	19/50 (38%)	17/50 (34%)	40/50 (80%)
Adjusted rates	34.7%	40.4%	38.6%	81.6%
Terminal rates	14/44 (32%)	18/46 (39%)	15/42 (36%)	36/45 (80%)
First incidence (days)	519	689	686	576
Life table tests	P<0.001	P=0.401	P=0.447	P<0.001
Logistic regression tests	P<0.001	P=0.371	P=0.526	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.338	P=0.500	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rates	7/50 (14%)	3/50 (6%)	8/50 (16%)	17/50 (34%)
Adjusted rates	15.9%	6.5%	17.1%	36.0%
Terminal rates	7/44 (16%)	3/46 (7%)	4/42 (10%)	15/45 (33%)
First incidence (days)	737 (T)	737 (T)	622	568
Life table tests	P<0.001	P=0.141N	P=0.479	P=0.022
Logistic regression tests	P<0.001	P=0.141N	P=0.506	P=0.019
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.159N	P=0.500	P=0.017

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rates	21/50 (42%)	20/50 (40%)	23/50 (46%)	45/50 (90%)
Adjusted rates	45.5%	42.6%	48.8%	90.0%
Terminal rates	19/44 (43%)	19/46 (41%)	18/42 (43%)	40/45 (89%)
First incidence (days)	519	689	622	568
Life table tests	P<0.001	P=0.428N	P=0.369	P<0.001
Logistic regression tests	P<0.001	P=0.451N	P=0.446	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.500N	P=0.420	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	13/50 (26%)	9/50 (18%)	7/50 (14%)	4/50 (8%)
Adjusted rates	28.2%	19.6%	16.7%	8.9%
Terminal rates	11/44 (25%)	9/46 (20%)	7/42 (17%)	4/45 (9%)
First incidence (days)	613	737 (T)	737 (T)	737 (T)
Life table tests	P=0.020N	P=0.204N	P=0.128N	P=0.016N
Logistic regression tests	P=0.018N	P=0.208N	P=0.096N	P=0.014N
Cochran-Armitage test	P=0.020N			
Fisher exact test		P=0.235N	P=0.105N	P=0.016N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rates	1/50 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rates	2.3%	6.5%	4.7%	2.2%
Terminal rates	1/44 (2%)	3/46 (7%)	1/42 (2%)	1/45 (2%)
First incidence (days)	737 (T)	737 (T)	704	737 (T)
Life table tests	P=0.418N	P=0.321	P=0.487	P=0.757N
Logistic regression tests	P=0.414N	P=0.321	P=0.506	P=0.757N
Cochran-Armitage test	P=0.422N			
Fisher exact test		P=0.309	P=0.500	P=0.753N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rates	14/50 (28%)	12/50 (24%)	9/50 (18%)	5/50 (10%)
Adjusted rates	30.4%	26.1%	20.9%	11.1%
Terminal rates	12/44 (27%)	12/46 (26%)	8/42 (19%)	5/45 (11%)
First incidence (days)	613	737 (T)	704	737 (T)
Life table tests	P=0.016N	P=0.362N	P=0.205N	P=0.020N
Logistic regression tests	P=0.014N	P=0.369N	P=0.157N	P=0.017N
Cochran-Armitage test	P=0.016N			
Fisher exact test		P=0.410N	P=0.171N	P=0.020N
Small Intestine (Jejunum): Carcinoma				
Overall rates	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rates	2.3%	0.0%	0.0%	6.7%
Terminal rates	1/44 (2%)	0/46 (0%)	0/42 (0%)	3/45 (7%)
First incidence (days)	737 (T)	–	–	737 (T)
Life table tests	P=0.055	P=0.491N	P=0.509N	P=0.314
Logistic regression tests	P=0.055	P=0.491N	P=0.509N	P=0.314
Cochran-Armitage test	P=0.053			
Fisher exact test		P=0.500N	P=0.500N	P=0.309

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
All Organs: Hemangioma or Hemangiosarcoma				
Overall rates	1/50 (2%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rates	2.3%	4.2%	4.4%	8.4%
Terminal rates	1/44 (2%)	1/46 (2%)	1/42 (2%)	2/45 (4%)
First incidence (days)	737 (T)	659	646	660
Life table tests	P=0.138	P=0.515	P=0.494	P=0.193
Logistic regression tests	P=0.111	P=0.494	P=0.494	P=0.170
Cochran-Armitage test	P=0.128			
Fisher exact test		P=0.500	P=0.500	P=0.181
All Organs: Malignant Lymphoma (Lymphocytic or Mixed)				
Overall rates	2/50 (4%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rates	4.4%	8.4%	4.8%	4.4%
Terminal rates	1/44 (2%)	3/46 (7%)	2/42 (5%)	2/45 (4%)
First incidence (days)	676	455	737 (T)	737 (T)
Life table tests	P=0.446N	P=0.360	P=0.681	P=0.685N
Logistic regression tests	P=0.478N	P=0.285	P=0.690N	P=0.691N
Cochran-Armitage test	P=0.450N			
Fisher exact test		P=0.339	P=0.691N	P=0.691N
All Organs: Malignant Lymphoma and Histiocytic Sarcoma				
Overall rates	3/50 (6%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rates	6.7%	8.4%	4.8%	6.7%
Terminal rates	2/44 (5%)	3/46 (7%)	2/42 (5%)	3/45 (7%)
First incidence (days)	676	455	737 (T)	737 (T)
Life table tests	P=0.539N	P=0.523	P=0.517N	P=0.651N
Logistic regression tests	P=0.567N	P=0.455	P=0.492N	P=0.651N
Cochran-Armitage test	P=0.546N			
Fisher exact test		P=0.500	P=0.500N	P=0.661N
All Organs: Benign Neoplasms				
Overall rates	25/50 (50%)	31/50 (62%)	21/50 (42%)	42/50 (84%)
Adjusted rates	53.1%	66.0%	47.7%	85.7%
Terminal rates	22/44 (50%)	30/46 (65%)	19/42 (45%)	38/45 (84%)
First incidence (days)	519	689	686	576
Life table tests	P<0.001	P=0.231	P=0.344N	P=0.002
Logistic regression tests	P<0.001	P=0.202	P=0.240N	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.157	P=0.274N	P<0.001
All Organs: Malignant Neoplasms				
Overall rates	14/50 (28%)	15/50 (30%)	12/50 (24%)	24/50 (48%)
Adjusted rates	31.1%	30.0%	25.4%	48.9%
Terminal rates	13/44 (30%)	11/46 (24%)	7/42 (17%)	20/45 (44%)
First incidence (days)	676	455	622	568
Life table tests	P=0.018	P=0.555	P=0.450N	P=0.047
Logistic regression tests	P=0.009	P=0.504	P=0.392N	P=0.036
Cochran-Armitage test	P=0.010			
Fisher exact test		P=0.500	P=0.410N	P=0.032

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rates	31/50 (62%)	37/50 (74%)	28/50 (56%)	46/50 (92%)
Adjusted rates	65.9%	74.0%	59.5%	92.0%
Terminal rates	28/44 (64%)	33/46 (72%)	23/42 (55%)	41/45 (91%)
First incidence (days)	519	455	622	568
Life table tests	P=0.003	P=0.251	P=0.434N	P=0.004
Logistic regression tests	P<0.001	P=0.173	P=0.295N	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.142	P=0.342N	P<0.001

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, epididymis, gallbladder, heart, kidney, larynx, liver, lung, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE C4a
Historical Incidence of Hepatocellular Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	16/50	7/50	21/50
dl-Amphetamine Sulfate	10/50	4/50	14/50
Overall Historical Incidence			
Total	312/1,366 (22.8%)	223/1,366 (16.3%)	485/1,366 (35.5%)
Standard deviation	13.8%	7.2%	14.3%
Range	4%-60%	3%-29%	10%-68%

[a] Data as of 20 August 1992

TABLE C4b
Historical Incidence of Renal Tubule Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	0/50	0/50	0/50
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	3/1,366 (0.2%)	1/1,366 (0.1%)	4/1,366 (0.3%)
Standard deviation	0.6%	0.4%	0.7%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 20 August 1992

TABLE C4c
Historical Incidence of Small Intestine Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma or Adenomatous Polyp	Carcinoma	Adenoma, Adenomatous Polyp, or Carinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	1/50	0/50	1/50
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	5/1,374 (0.4%)	7/1,374 (0.5%)	12/1,374 (0.9%)
Standard deviation	1.0%	1.0%	1.0%
Range	0%-4%	0%-4%	0%-4%

[a] Data as of 20 August 1992

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	9	10	10	10
Early deaths				
Accidental deaths	1			
Moribund	2	2	3	1
Natural deaths	3	2	5	4
Survivors				
Terminal sacrifice	44	46	42	45
Missing	1			
Animals examined microscopically	59	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(9)	(10)	(10)	(10)
Basophilic focus		1 (10%)		3 (30%)
Eosinophilic focus				2 (20%)
Mixed cell focus		1 (10%)		
Vacuolization, cytoplasmic	3 (33%)		2 (20%)	1 (10%)
Salivary gland	(9)			(10)
Infiltration cellular, lymphocyte	1 (11%)			1 (10%)
Cardiovascular System				
None				
Endocrine System				
Adrenal gland, cortex	(9)			(10)
Cytologic alteration	1 (11%)			
Cystic, degeneration	1 (11%)			
Hypertrophy				1 (10%)
Thyroid	(9)			(10)
Cyst	1 (11%)			
Parathyroid	(8)			(7)
Cyst				1 (14%)
General Body System				
None				
Genital System				
Preputial gland	(9)	(4)	(10)	(10)
Duct, dilatation	2 (22%)			
Testes	(9)			(10)
Bilateral, seminiferous tubule, atrophy				1 (10%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Hematopoietic System				
Lymph node, mesenteric	(9)		(1)	(8)
Hemmorrhage			1 (100%)	
Thymus	(9)			(10)
Hyperplasia, epithelial cell	1 (11%)			
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
Brain	(9)			(10)
Mineralization	5 (56%)			7 (70%)
Respiratory System				
Lung	(9)	(3)	(2)	(10)
Alveolar epithelium, hyperplasia	1 (11%)			1 (10%)
Special Senses System				
None				
Urinary System				
Kidney	(9)			(10)
Renal tubule, regeneration	4 (44%)			3 (30%)
Urinary bladder	(9)			(10)
Infiltration cellular, lymphocyte				1 (10%)
2-Year Study				
Alimentary System				
Gallbladder	(45)	(47)	(46)	(47)
Hyperplasia				1 (2%)
Inflammation, acute				2 (4%)
Intestine large, colon	(48)	(50)	(50)	(49)
Diverticulum		1 (2%)		
Intestine large, cecum	(48)	(49)	(48)	(48)
Hyperplasia, lymphoid		1 (2%)		
Intestine small, duodenum	(48)	(48)	(47)	(49)
Hyperplasia, lymphoid		1 (2%)		
Inflammation, acute, focal				1 (2%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Intestine small, jejunum	(49)	(49)	(49)	(49)
Hyperplasia, lymphoid				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal		1 (2%)	1 (2%)	
Basophilic focus	7 (14%)	5 (10%)	4 (8%)	9 (18%)
Clear cell focus	8 (16%)	5 (10%)	5 (10%)	4 (8%)
Cytomegaly, focal		1 (2%)		
Developmental malformation	1 (2%)			
Eosinophilic focus	13 (26%)	12 (24%)	10 (20%)	28 (56%)
Fatty change, focal		1 (2%)	3 (6%)	4 (8%)
Hematopoietic cell proliferation		1 (2%)		
Infarct, chronic	1 (2%)			
Inflammation, chronic, focal	1 (2%)		1 (2%)	
Leukocytosis	1 (2%)		1 (2%)	
Mixed cell focus	3 (6%)	5 (10%)	2 (4%)	1 (2%)
Necrosis, focal			4 (8%)	3 (6%)
Mesentery	(3)	(1)		
Fat, necrosis, focal	3 (100%)			
Pancreas	(49)	(50)	(50)	(50)
Cyst			1 (2%)	
Acinar cell, atrophy		1 (2%)		1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	3 (6%)	1 (2%)	
Inflammation, chronic active, focal	1 (2%)			
Ulcer, focal	1 (2%)	1 (2%)	1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Hyperplasia, focal				1 (2%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	1 (2%)			
Inflammation, acute, focal			1 (2%)	
Inflammation, chronic active, focal			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule		1 (2%)		
Atrophy				1 (2%)
Hemorrhage, focal				1 (2%)
Hyperplasia, focal	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Hypertrophy, focal	1 (2%)	1 (2%)	3 (6%)	4 (8%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia, focal			1 (2%)	1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		1 (2%)	

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Endocrine System (continued)				
Parathyroid gland	(41)	(43)	(42)	(43)
Cyst			1 (2%)	
Inflammation, chronic, focal	1 (2%)			
Pituitary gland	(49)	(48)	(48)	(48)
Pars distalis, cyst	1 (2%)	2 (4%)		
Pars distalis, hyperplasia, focal			2 (4%)	
Pars nervosa, cyst		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, dilatation, focal			1 (2%)	1 (2%)
Follicular cell, hyperplasia, focal	3 (6%)	1 (2%)		5 (10%)
Follicular cell, hypertrophy, focal			1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	2 (4%)	2 (4%)		
Inflammation, chronic, focal	1 (2%)	2 (4%)		
Preputial gland	(29)	(23)	(23)	(21)
Inflammation, chronic active	2 (7%)		2 (9%)	1 (5%)
Duct, dilatation	27 (93%)	23 (100%)	22 (96%)	21 (100%)
Prostate	(50)	(49)	(50)	(50)
Dilatation		1 (2%)		
Hyperplasia, focal	2 (4%)	4 (8%)	6 (12%)	2 (4%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	2 (4%)	1 (2%)	1 (2%)	
Inflammation, acute	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Angiectasis, focal			1 (2%)	
Atrophy		1 (2%)		
Atrophy, focal		1 (2%)		
Degeneration, focal	1 (2%)		1 (2%)	
Seminiferous tubule, degeneration, focal	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis, focal		1 (2%)		1 (2%)
Hyperplasia	1 (2%)	1 (2%)		
Lymph node	(4)	(1)	(2)	(3)
Hyperplasia, lymphoid			1 (50%)	
Bronchial, hyperplasia, lymphoid	1 (25%)			
Mediastinal, congestion				1 (33%)
Renal, hyperplasia, lymphoid	1 (25%)			

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node, mandibular	(46)	(49)	(49)	(48)
Cyst			1 (2%)	1 (2%)
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	4 (9%)	4 (8%)	4 (8%)	1 (2%)
Hyperplasia, plasma cell		1 (2%)	1 (2%)	
Infiltration cellular, histiocyte				1 (2%)
Lymph node, mesenteric	(48)	(49)	(50)	(50)
Hemorrhage	3 (6%)	3 (6%)	2 (4%)	
Hyperplasia, lymphoid	2 (4%)	4 (8%)		1 (2%)
Hyperplasia, plasma cell	1 (2%)			
Necrosis	1 (2%)			
Spleen	(49)	(50)	(50)	(50)
Developmental malformation		1 (2%)		
Hematopoietic cell proliferation	3 (6%)	3 (6%)	3 (6%)	4 (8%)
Hyperplasia, lymphoid	2 (4%)	4 (8%)	2 (4%)	
Lymphocyte, necrosis, focal	1 (2%)			
Thymus	(49)	(45)	(46)	(42)
Cyst	2 (4%)	2 (4%)	3 (7%)	2 (5%)
Necrosis	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic active, focal	1 (2%)	3 (6%)		
Subcutaneous tissue, abscess			1 (2%)	
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage, focal	1 (2%)		1 (2%)	
Mineralization, focal	36 (72%)	42 (84%)	43 (86%)	43 (86%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage, focal				1 (2%)
Infiltration cellular, focal, histiocyte		2 (4%)	1 (2%)	
Leukocytosis	1 (2%)		1 (2%)	
Alveolar epithelium, hyperplasia, focal	5 (10%)	2 (4%)	3 (6%)	2 (4%)
Interstitium, inflammation, focal	4 (8%)	1 (2%)	3 (6%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Congestion	1 (2%)			1 (2%)
Inflammation, acute, focal			3 (6%)	1 (2%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218
(continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Special Senses System				
Ear	(2)			
Middle ear, inflammation, chronic active, focal	1 (50%)			
Eye	(1)	(2)	(2)	
Cornea, inflammation, chronic		1 (50%)	1 (50%)	
Lens, cataract		1 (50%)		
Zymbal's gland	(39)	(34)	(42)	(37)
Inflammation, chronic active			1 (2%)	
Duct, dilatation			1 (2%)	1 (3%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct, chronic, focal			2 (4%)	
Metaplasia, focal, osseous				1 (2%)
Mineralization, focal			1 (2%)	
Nephropathy	25 (50%)	20 (40%)	30 (60%)	19 (38%)
Renal tubule, dilatation, focal	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Dilatation	1 (2%)		1 (2%)	
Metaplasia, focal, osseous				1 (2%)
Transitional epithelium, hyperplasia, focal	1 (2%)			

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF C.I. DIRECT BLUE 218

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Accidental deaths				1
Moribund	8	8	1	5
Natural deaths	4	2	2	5
Survivors				
Terminal sacrifice	37	40	46	38
Missing	1		1	1
Animals examined microscopically	59	60	59	59
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatocellular adenoma		1 (10%)	1 (10%)	1 (10%)
Hepatocellular carcinoma				1 (10%)
Cardiovascular System				
None				
Endocrine System				
Adrenal medulla	(10)			(10)
Pheochromocytoma	1 (10%)			
General Body System				
None				
Genital System				
None				
Hematopoietic System				
None				
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Respiratory System				
Lung	(10)	(1)		(10)
Alveolar/bronchiolar adenoma	1 (10%)			
Special Senses System				
None				
Urinary System				
None				
Systemic Lesions				
None				
2-Year Study				
Alimentary System				
Gallbladder	(47)	(45)	(46)	(42)
Intestine large, cecum	(47)	(50)	(48)	(45)
Intestine small, duodenum	(46)	(50)	(48)	(46)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Intestine small, jejunum	(47)	(49)	(48)	(45)
Carcinoma			1 (2%)	
Intestine small, ileum	(48)	(50)	(48)	(45)
Carcinoma				1 (2%)
Liver	(49)	(50)	(49)	(49)
Hemangioma	1 (2%)			
Hemangiosarcoma	1 (2%)	3 (6%)		1 (2%)
Hemangiosarcoma, metastatic, skin		1 (2%)		
Hepatoblastoma				1 (2%)
Hepatocellular carcinoma	5 (10%)	5 (10%)	6 (12%)	12 (24%)
Hepatocellular adenoma	6 (12%)	8 (16%)	9 (18%)	6 (12%)
Hepatocellular adenoma, multiple	1 (2%)	4 (8%)	8 (16%)	35 (71%)
Hepatocholangiocarcinoma		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Osteosarcoma, metastatic, bone		1 (2%)		
Osteosarcoma, metastatic, nose			1 (2%)	
Sarcoma stromal, metastatic, uterus	1 (2%)			
Mesentery	(6)	(5)	(3)	(5)
Hepatocholangiocarcinoma, metastatic, liver		1 (20%)		
Histiocytic sarcoma		1 (20%)		
Pancreas	(49)	(50)	(48)	(49)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Salivary glands	(49)	(50)	(49)	(48)
Stomach, forestomach	(49)	(50)	(48)	(49)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Squamous cell papilloma				1 (2%)
Stomach, glandular	(49)	(50)	(48)	(49)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Cardiovascular System				
Heart	(49)	(50)	(49)	(49)
Histiocytic sarcoma		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(49)	(48)	(48)
Adenoma				1 (2%)
Adrenal medulla	(49)	(49)	(48)	(47)
Pheochromocytoma benign		1 (2%)	2 (4%)	
Pituitary gland	(48)	(50)	(49)	(45)
Pars distalis, adenoma	2 (4%)	6 (12%)	2 (4%)	
Thyroid gland	(49)	(50)	(49)	(49)
Follicular cell, adenoma		1 (2%)		1 (2%)
Follicular cell, carcinoma		1 (2%)		
General Body System				
Tissue NOS	(1)	(2)		(1)
Carcinoma, metastatic, thyroid gland		1 (50%)		
Sarcoma, metastatic, skeletal muscle				1 (100%)
Genital System				
Ovary	(47)	(50)	(48)	(49)
Choriocarcinoma				1 (2%)
Cystadenoma	2 (4%)	1 (2%)		1 (2%)
Granulosa cell tumor benign		1 (2%)	2 (4%)	
Hemangioma			1 (2%)	1 (2%)
Teratoma benign	1 (2%)		1 (2%)	1 (2%)
Thecoma benign			1 (2%)	
Uterus	(49)	(50)	(49)	(49)
Deciduoma benign				1 (2%)
Hemangioma				1 (2%)
Hemangiosarcoma				1 (2%)
Hemangiosarcoma, metastatic, bone marrow		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Leiomyoma	1 (2%)			1 (2%)
Polyp stromal			1 (2%)	1 (2%)
Sarcoma stromal	1 (2%)	1 (2%)	1 (2%)	
Hematopoietic System				
Bone marrow	(49)	(50)	(49)	(49)
Vertebral, hemangiosarcoma		1 (2%)		
Lymph node	(9)	(10)	(8)	(7)
Bronchial, hepatocellular carcinoma, metastatic, liver		1 (10%)		
Bronchial, osteosarcoma, metastatic, bone		1 (10%)		
Iliac, sarcoma stromal, metastatic, uterus	1 (11%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node (continued)				
Lumbar, hepatocholangiocarcinoma, metastatic, liver		1 (10%)		
Mediastinal, hepatocholangiocarcinoma, metastatic, liver		1 (10%)		
Renal, hepatocholangiocarcinoma, metastatic, liver		1 (10%)		
Lymph node, mandibular	(49)	(48)	(48)	(47)
Lymph node, mesenteric	(46)	(48)	(47)	(46)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Spleen	(48)	(50)	(49)	(49)
Hemangiosarcoma		1 (2%)		
Thymus	(46)	(48)	(48)	(46)
Integumentary System				
Mammary gland	(49)	(49)	(49)	(48)
Adenoma	1 (2%)			
Skin	(49)	(50)	(49)	(49)
Squamous cell carcinoma				1 (2%)
Subcutaneous tissue, hemangiosarcoma		2 (4%)		
Subcutaneous tissue, sarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant			1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(49)	(50)	(49)	(49)
Dorsal, osteosarcoma		1 (2%)		
Vertebra, hemangiosarcoma, extension		1 (2%)		
Skeletal muscle		(3)		(1)
Back, hemangiosarcoma, extension		1 (33%)		
Back, sarcoma				1 (100%)
Diaphragm, hepatocholangiocarcinoma, metastatic, liver		1 (33%)		
Nervous System				
None				
Respiratory System				
Lung	(49)	(50)	(49)	(49)
Alveolar/bronchiolar adenoma	5 (10%)	5 (10%)	4 (8%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple		2 (4%)		
Hepatocellular carcinoma, metastatic, liver	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Osteosarcoma, metastatic, bone		1 (2%)		
Osteosarcoma, metastatic, nose			1 (2%)	
Sarcoma stromal, metastatic, uterus	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Respiratory System (continued)				
Nose	(49)	(50)	(49)	(49)
Osteosarcoma			1 (2%)	
Trachea	(49)	(50)	(49)	(49)
Special Senses System				
Harderian gland	(3)		(2)	
Adenoma	3 (100%)		2 (100%)	
Urinary System				
Kidney	(49)	(50)	(49)	(49)
Bilateral, osteosarcoma, metastatic, bone		1 (2%)		
Urinary bladder	(48)	(50)	(48)	(49)
Histiocytic sarcoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(49)	(50)	(49)	(49)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant histiocytic	1 (2%)	2 (4%)		1 (2%)
Lymphoma malignant lymphocytic	4 (8%)	7 (14%)	5 (10%)	2 (4%)
Lymphoma malignant mixed	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Lymphoma malignant undifferentiated cell	1 (2%)		1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	29	35	34	46
Total primary neoplasms	37	59	52	78
Total animals with benign neoplasms	20	21	26	42
Total benign neoplasms	23	29	33	53
Total animals with malignant neoplasms	14	24	17	24
Total malignant neoplasms	14	30	19	25
Total animals with metastatic neoplasms	2	6	3	2
Total metastatic neoplasms	4	20	4	2

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm

Number of Days on Study	0 8 8	1 8 4	3 6 8	4 5 6	5 1 8	5 8 0	6 1 3	6 1 7	6 2 7	6 4 4	6 8 8	7 0 1	7 2 8	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4
Carcass ID Number	2 5 6 1	2 5 9 1	2 6 4 1	3 0 0 1	2 4 1 1	2 5 4 1	2 8 7 1	2 7 5 1	2 8 5 1	2 7 7 1	2 7 0 1	2 7 9 1	2 6 3 1	2 4 2 1	2 4 3 1	2 4 4 1	2 4 5 1	2 4 6 1	2 4 7 1	2 4 8 1	2 4 9 1	2 5 0 1	2 5 1 1	2 5 2 1	2 5 3 1
Alimentary System																									
Esophagus	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	A	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+		+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma									X																
Hemangiosarcoma						X																			
Hepatocellular carcinoma														X											
Hepatocellular adenoma												X						X							
Hepatocellular adenoma, multiple																									
Sarcoma stromal, metastatic, uterus											X														
Mesentery															+	+									
Pancreas	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+		+	+	+	+	+	+	+	+	+	+	M	+	M	+	M	+	+	M	M	M	M
Pituitary gland	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Pars distalis, adenoma														X			X								
Thyroid gland	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS										+															
Genital System																									
Clitoral gland																									
Ovary	+	+	+		+	+	M	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Teratoma benign		X																							
Uterus	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																								X	
Sarcoma stromal											X														

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	
Carcass ID Number	2 5 5 1	2 5 7 1	2 5 8 1	2 6 0 1	2 6 1 1	2 6 2 1	2 6 5 1	2 6 6 1	2 6 7 1	2 6 8 1	2 6 9 1	2 7 1 1	2 7 3 1	2 7 4 1	2 7 6 1	2 7 8 1	2 8 0 1	2 8 1 1	2 8 2 1	2 8 3 1	2 8 4 1	2 8 6 1	2 8 8 1	2 8 9 1	2 9 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																										1
Hemangiosarcoma																										1
Hepatocellular carcinoma							X						X	X						X						5
Hepatocellular adenoma									X				X	X							X					6
Hepatocellular adenoma, multiple			X																							1
Sarcoma stromal, metastatic, uterus																										1
Mesentery											+	+								+	+					6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	M	M	+	+	M	+	+	M	+	M	+	+	M	+	M	+	+	+	M	33
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
Tissue NOS																										1
Genital System																										
Clitoral gland				+																						1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cystadenoma										X				X												2
Teratoma benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyoma																										1
Sarcoma stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	0 8 8	1 8 4	3 6 8	5 1 8	5 8 0	6 1 3	6 1 7	6 2 7	6 4 4	6 8 8	7 0 1	7 2 8	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4
Carcass ID Number	2 5 6 1	2 5 9 1	2 6 4 1	2 4 1 1	2 5 4 1	2 8 7 1	2 7 5 1	2 8 5 1	2 7 7 1	2 7 0 1	2 7 9 1	2 6 3 1	2 4 2 1	2 4 3 1	2 4 4 1	2 4 5 1	2 4 6 1	2 4 7 1	2 4 8 1	2 4 9 1	2 5 0 1	2 5 1 1	2 5 2 1	2 5 3 1
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node										+		+	+						+			+	+	
Iliac, sarcoma stromal, metastatic, uterus										X														
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X			
Hepatocellular carcinoma, metastatic, liver																								
Sarcoma stromal, metastatic, uterus										X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Ear			+				+																	
Eye																								
Harderian gland																								
Adenoma																								
Zymbal's gland				+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic				X																				
Lymphoma malignant lymphocytic														X								X	X	
Lymphoma malignant mixed																								
Lymphoma malignant undifferentiated cell type									X															

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 0 ppm
(continued)

Number of Days on Study	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	
Carcass ID Number	2 5 5 1	2 5 7 1	2 5 8 1	2 6 0 1	2 6 1 1	2 6 2 1	2 6 5 1	2 6 6 1	2 6 7 1	2 6 8 1	2 6 9 1	2 7 1 1	2 7 3 1	2 7 4 1	2 7 6 1	2 7 8 1	2 8 0 1	2 8 1 1	2 8 2 1	2 8 3 1	2 8 4 1	2 8 6 1	2 8 8 1	2 8 9 1	2 9 0 1	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node		+												+					+							9
Iliac, sarcoma stromal, metastatic, uterus																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma			X																							1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma				X	X		X								X											5
Hepatocellular carcinoma, metastatic, liver													X													1
Sarcoma stromal, metastatic, uterus																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Ear																										2
Eye							+				+															2
Harderian gland							+				+					+										3
Adenoma							X				X					X										3
Zymbal's gland	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	38
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																								X		4
Lymphoma malignant mixed		X																								1
Lymphoma malignant undifferentiated cell type																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm

Number of Days on Study	5 2 2	5 5 4	5 9 0	6 3 9	6 3 9	6 4 8	6 4 8	6 6 9	6 7 9	6 9 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3
Carcass ID Number	3 3 2 1	3 2 4 1	3 1 3 1	3 0 2 1	3 4 1 1	3 4 0 1	3 4 5 1	3 1 6 1	3 4 2 1	3 4 8 1	3 0 1 1	3 0 3 1	3 0 4 1	3 0 5 1	3 0 6 1	3 0 7 1	3 0 8 1	3 0 9 1	3 1 0 1	3 1 1 1	3 1 2 1	3 1 4 1	3 1 5 1	3 1 7 1	3 1 8 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																									
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X						X								
Hemangiosarcoma, metastatic, skin																									
Hepatocellular carcinoma						X																			X
Hepatocellular adenoma															X					X					
Hepatocellular adenoma, multiple																	X		X						
Hepatocholangiocarcinoma																									
Histiocytic sarcoma		X																							
Osteosarcoma, metastatic, bone									X																
Mesentery		+			+																				
Hepatocholangiocarcinoma, metastatic, liver																									
Histiocytic sarcoma		X																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Endocrine System																									
Adrenal cortex	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																X						X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
Follicular cell, carcinoma		X																							

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	
Carcass ID Number	3 1 9 1	3 2 0 1	3 2 1 1	3 2 2 1	3 2 3 1	3 2 5 1	3 2 6 1	3 2 7 1	3 2 8 1	3 2 9 1	3 3 0 1	3 3 1 1	3 3 3 1	3 3 4 1	3 3 5 1	3 3 6 1	3 3 7 1	3 3 8 1	3 3 9 1	3 4 3 1	3 4 4 1	3 4 6 1	3 4 7 1	3 4 9 1	3 5 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma															X											3
Hemangiosarcoma, metastatic, skin											X															1
Hepatocellular carcinoma						X				X					X											5
Hepatocellular adenoma						X			X			X			X				X	X						8
Hepatocellular adenoma, multiple				X																				X		4
Hepatocholangiocarcinoma	X																									1
Histiocytic sarcoma																										1
Osteosarcoma, metastatic, bone																										1
Mesentery	+				+										+											5
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																			X							1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	M	M	M	M	M	+	M	+	M	+	M	+	+	M	34
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma														X							X		X			6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma														X												1
Follicular cell, carcinoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	5 2 2	5 5 4	5 9 0	6 3 9	6 3 9	6 4 8	6 4 8	6 6 9	6 7 9	6 9 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3
Carcass ID Number	3 3 2 1	3 2 4 1	3 1 3 1	3 0 2 1	3 4 1 1	3 4 0 1	3 4 5 1	3 1 6 1	3 4 2 1	3 4 8 1	3 0 1 1	3 0 3 1	3 0 4 1	3 0 5 1	3 0 6 1	3 0 7 1	3 0 8 1	3 0 9 1	3 1 0 1	3 1 1 1	3 1 2 1	3 1 4 1	3 1 5 1	3 1 7 1	3 1 8 1
General Body System																									
Tissue NOS		+																	+						
Carcinoma, metastatic, thyroid gland		X																							
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																		X							
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, bone marrow	X																								
Histiocytic sarcoma		X																							
Sarcoma stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vertebral, hemangiosarcoma	X																								
Lymph node			+			+	+		+										+						
Bronchial, hepatocellular carcinoma, metastatic, liver						X																			
Bronchial, osteosarcoma, metastatic, bone									X																
Lumbar, hepatocholangiocarcinoma, metastatic, liver																									
Mediastinal, hepatocholangiocarcinoma, metastatic, liver																									
Renal, hepatocholangiocarcinoma, metastatic, liver																									
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Lymph node, mesenteric	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma										X															
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma										X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dorsal, osteosarcoma									X																
Vertebra, hemangiosarcoma, extension	X																								
Skeletal muscle	+			+																					
Back, hemangiosarcoma, extension	X																								
Diaphragm, hepatocholangiocarcinoma, metastatic, liver																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	
Carcass ID Number	3 1 9 1	3 2 0 1	3 2 1 1	3 2 2 1	3 2 3 1	3 2 5 1	3 2 6 1	3 2 7 1	3 2 8 1	3 2 9 1	3 3 0 1	3 3 1 1	3 3 3 1	3 3 4 1	3 3 5 1	3 3 6 1	3 3 7 1	3 3 8 1	3 3 9 1	3 4 3 1	3 4 4 1	3 4 6 1	3 4 7 1	3 4 9 1	3 5 0 1	Total Tissues/ Tumors
General Body System																										
Tissue NOS																										2
Carcinoma, metastatic, thyroid gland																										1
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																										1
Granulosa cell tumor benign							X																			1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, bone marrow																										1
Histiocytic sarcoma																										1
Sarcoma stromal																							X			1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Vertebral, hemangiosarcoma																										1
Lymph node	+								+	+												+	+			10
Bronchial, hepatocellular carcinoma, metastatic, liver																										1
Bronchial, osteosarcoma, metastatic, bone																										1
Lumbar, hepatocholangiocarcinoma, metastatic, liver	X																									1
Mediastinal, hepatocholangiocarcinoma, metastatic, liver	X																									1
Renal, hepatocholangiocarcinoma, metastatic, liver	X																									1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma											X															2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Dorsal, osteosarcoma																										1
Vertebra, hemangiosarcoma, extension																										1
Skeletal muscle	+																									3
Back, hemangiosarcoma, extension																										1
Diaphragm, hepatocholangiocarcinoma, metastatic, liver	X																									1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	5 2 2	5 5 4	5 9 0	6 3 9	6 3 9	6 4 8	6 4 8	6 6 9	6 7 9	6 9 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3
Carcass ID Number	3 3 2 1	3 2 4 1	3 1 3 1	3 0 2 1	3 4 1 1	3 4 0 1	3 4 5 1	3 1 6 1	3 4 2 1	3 4 8 1	3 0 1 1	3 0 3 1	3 0 4 1	3 0 5 1	3 0 6 1	3 0 7 1	3 0 8 1	3 0 9 1	3 1 0 1	3 1 1 1	3 1 2 1	3 1 4 1	3 1 5 1	3 1 7 1	3 1 8 1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																		+							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X								
Alveolar/bronchiolar adenoma, multiple																						X	X		
Hepatocellular carcinoma, metastatic, liver						X																			
Hepatocholangiocarcinoma, metastatic, liver																									
Histiocytic sarcoma		X																							
Osteosarcoma, metastatic, bone									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye											+														
Zymbal's gland	+	M	M	I	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	M	+	M	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, osteosarcoma, metastatic, bone									X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymphoma malignant histiocytic								X																	
Lymphoma malignant lymphocytic			X		X		X												X						
Lymphoma malignant mixed																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 1,000 ppm
(continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	
Carcass ID Number	3 1 9 1	3 2 0 1	3 2 1 1	3 2 2 1	3 2 3 1	3 2 5 1	3 2 6 1	3 2 7 1	3 2 8 1	3 2 9 1	3 3 0 1	3 3 1 1	3 3 3 1	3 3 4 1	3 3 5 1	3 3 6 1	3 3 7 1	3 3 8 1	3 3 9 1	3 4 3 1	3 4 4 1	3 4 6 1	3 4 7 1	3 4 9 1	3 5 0 1	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma															X	X							X	X		5
Alveolar/bronchiolar adenoma, multiple																										2
Hepatocellular carcinoma, metastatic, liver																										1
Hepatocholangiocarcinoma, metastatic, liver	X																									1
Histiocytic sarcoma																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear		+																								1
Eye																										1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	M	+	+	M	+	+	+	38
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, osteosarcoma, metastatic, bone																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant histiocytic																X										2
Lymphoma malignant lymphocytic							X														X			X		7
Lymphoma malignant mixed										X												X				2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm

Number of Days on Study	5 3 2	6 7 6	7 0 4	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2
Carcass ID Number	3 7 3 1	3 7 6 1	3 8 8 1	3 6 1 1	3 6 2 1	3 6 3 1	3 6 4 1	3 6 5 1	3 6 6 1	3 6 7 1	3 6 8 1	3 6 9 1	3 7 0 1	3 7 1 1	3 7 2 1	3 7 4 1	3 7 5 1	3 7 7 1	3 7 8 1	3 7 9 1	3 8 0 1	3 8 1 1	3 8 2 1	3 8 3 1
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X				
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma							X		X									X			X			
Hepatocellular adenoma		X			X												X	X						
Hepatocellular adenoma, multiple									X						X	X								
Osteosarcoma, metastatic, nose		X																						
Mesentery								+																
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign														X										
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	A	+	+	M	+	+	+	M	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								
Genital System																								
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																								
Hemangioma																								
Teratoma benign																								
Thecoma benign																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal														X										
Sarcoma stromal						X																		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 4 2	7 4 2	7 4 2	7 4 2	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	
Carcass ID Number	3 8 4 1	3 8 5 1	3 8 6 1	3 8 7 1	3 8 9 1	3 9 0 1	3 9 1 1	3 9 2 1	3 9 3 1	3 9 4 1	3 9 5 1	3 9 6 1	3 9 7 1	3 9 8 1	3 9 9 1	4 0 0 1	4 0 2 1	4 0 3 1	4 0 4 1	4 0 5 1	4 0 6 1	4 0 7 1	4 0 8 1	4 0 9 1	4 1 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma																		X				X				6
Hepatocellular adenoma				X	X					X							X							X		9
Hepatocellular adenoma, multiple		X										X		X							X		X			8
Osteosarcoma, metastatic, nose																										1
Mesentery				+											+											3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign																						X				2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	M	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma							X			X																2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Granulosa cell tumor benign														X			X									2
Hemangioma									X																	1
Teratoma benign			X																							1
Thecoma benign														X												1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal																										1
Sarcoma stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	5 3 2	6 7 6	7 0 4	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2
Carcass ID Number	3 7 3 1	3 7 6 1	3 8 8 1	3 6 1 1	3 6 2 1	3 6 3 1	3 6 4 1	3 6 5 1	3 6 6 1	3 6 7 1	3 6 8 1	3 6 9 1	3 7 0 1	3 7 1 1	3 7 2 1	3 7 4 1	3 7 5 1	3 7 7 1	3 7 8 1	3 7 9 1	3 8 0 1	3 8 1 1	3 8 2 1	3 8 3 1
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+						+		+		+							+		+		
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	I	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma															X									
Subcutaneous tissue, schwannoma malignant																								
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X																		X	
Hepatocellular carcinoma, metastatic, liver																					X			
Osteosarcoma, metastatic, nose		X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma		X																						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye																								
Harderian gland							+																	
Adenoma							X																	
Zymbal's gland	+	M	M	+	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	M	+	+
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic			X																	X		X		
Lymphoma malignant mixed									X				X											
Lymphoma malignant undifferentiated cell type	X																							

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 3,000 ppm
(continued)

Number of Days on Study	7 4 2	7 4 2	7 4 2	7 4 2	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	
Carcass ID Number	3 8 4 1	3 8 5 1	3 8 6 1	3 8 7 1	3 8 9 1	3 9 0 1	3 9 1 1	3 9 2 1	3 9 3 1	3 9 4 1	3 9 5 1	3 9 6 1	3 9 7 1	3 9 8 1	3 9 9 1	4 0 0 1	4 0 2 1	4 0 3 1	4 0 4 1	4 0 5 1	4 0 6 1	4 0 7 1	4 0 8 1	4 0 9 1	4 1 0 1	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node			+										+													8
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, sarcoma																										1
Subcutaneous tissue, schwannoma malignant					X																					1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma						X												X								4
Hepatocellular carcinoma, metastatic, liver																						X				2
Osteosarcoma, metastatic, nose																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Osteosarcoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye			+																							1
Harderian gland			+																							2
Adenoma			X																							2
Zymbal's gland	M	M	M	+	+	+	M	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	37
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant lymphocytic			X										X													5
Lymphoma malignant mixed																										2
Lymphoma malignant undifferentiated cell type																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm

Number of Days on Study	0 3 8	1 0 8	4 2 7	5 0 4	5 4 1	5 6 7	6 8 9	6 9 4	7 0 0	7 1 5	7 2 1	7 3 9	7 3 9	7 3 9	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2
Carcass ID Number	4 6 2 1	4 5 5 1	4 4 6 1	4 3 8 1	4 4 2 1	4 2 7 1	4 5 1 1	4 6 4 1	4 4 0 1	4 6 0 1	4 6 3 1	4 2 1 1	4 2 2 1	4 2 3 1	4 2 4 1	4 2 5 1	4 2 6 1	4 2 8 1	4 2 9 1	4 3 0 1	4 3 1 1	4 3 2 1	4 3 3 1	4 3 4 1
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	A	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																			X					
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma												X												
Hepatoblastoma																		X						
Hepatocellular carcinoma							X									X					X			
Hepatocellular adenoma					X	X			X							X								
Hepatocellular adenoma, multiple							X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X
Mesentery			+							+														+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																								
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																						X		
General Body System																								
Tissue NOS									+															
Sarcoma, metastatic, skeletal muscle									X															
Genital System																								
Clitoral gland																								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Choriocarcinoma		X																						
Cystadenoma																								
Hemangioma																								
Teratoma benign																		X						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deciduoma benign		X																						
Hemangioma															X									
Hemangiosarcoma														X										
Leiomyoma																								
Polyp stromal																								

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	
Carcass ID Number	4 3 5 1	4 3 6 1	4 3 7 1	4 3 9 1	4 4 1 1	4 4 3 1	4 4 4 1	4 4 5 1	4 4 7 1	4 4 8 1	4 4 9 1	4 5 0 1	4 5 2 1	4 5 3 1	4 5 4 1	4 5 6 1	4 5 7 1	4 5 8 1	4 5 9 1	4 6 1 1	4 6 5 1	4 6 6 1	4 6 7 1	4 6 9 1	4 7 0 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	M	+	+	+	42
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Carcinoma																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Hepatoblastoma																										1
Hepatocellular carcinoma	X						X	X				X							X			X	X	X	X	12
Hepatocellular adenoma				X																				X		6
Hepatocellular adenoma, multiple		X	X		X	X		X	X	X	X	X	X	X	X	X	X	X		X	X		X		X	35
Mesentery	+	+																								5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																							X			1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																		X								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	M	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	M	40
Pituitary gland	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma																										1
General Body System																										
Tissue NOS																										1
Sarcoma, metastatic, skeletal muscle																										1
Genital System																										
Clitoral gland															+											1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Choriocarcinoma																										1
Cystadenoma						X																				1
Hemangioma																		X								1
Teratoma benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Deciduoma benign																										1
Hemangioma																										1
Hemangiosarcoma																										1
Leiomyoma				X																						1
Polyp stromal		X																								1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	0 3 8	1 0 8	4 2 7	5 0 4	5 4 1	5 6 7	6 8 9	6 9 4	7 0 0	7 1 5	7 2 1	7 3 9	7 3 9	7 3 9	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2
Carcass ID Number	4 6 2 1	4 5 5 1	4 4 6 1	4 3 8 1	4 4 2 1	4 2 7 1	4 5 1 1	4 6 4 1	4 4 0 1	4 6 0 1	4 6 3 1	4 2 1 1	4 2 2 1	4 2 3 1	4 2 4 1	4 2 5 1	4 2 6 1	4 2 8 1	4 3 9 1	4 3 0 1	4 3 1 1	4 3 2 1	4 3 3 1	4 3 4 1
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node								+	+	+														+
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																								
Subcutaneous tissue, schwannoma malignant											X													
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle									+															
Back, sarcoma									X															
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								
Hepatocellular carcinoma, metastatic, liver																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Zymbal's gland				+	+	M	+	M	+	+	+	+	M	+	+	+	+	M	M	+	+	+	M	+
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																
Lymphoma malignant lymphocytic																								X
Lymphoma malignant mixed																								

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218: 10,000 ppm
(continued)

Number of Days on Study	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	7 4 2	
Carcass ID Number	4 3 5 1	4 3 6 1	4 3 7 1	4 3 9 1	4 4 1 1	4 4 3 1	4 4 4 1	4 4 5 1	4 4 7 1	4 4 8 1	4 4 9 1	4 5 0 1	4 5 2 1	4 5 3 1	4 5 4 1	4 5 6 1	4 5 7 1	4 5 8 1	4 5 9 1	4 6 1 1	4 6 5 1	4 6 6 1	4 6 7 1	4 6 9 1	4 7 0 1	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+												+		+											7
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma					X																					1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skeletal muscle																										1
Back, sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma						X																				1
Hepatocellular carcinoma, metastatic, liver																			X							1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Zymbal's gland	+	M	+	+	+	+	M	M	+	+	M	+	+	+	+	+	M	+	+	M	+	+	M	M	+	32
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic	X																									2
Lymphoma malignant mixed													X		X											2

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Harderian Gland: Adenoma				
Overall rates[a]	3/49 (6%)	0/50 (0%)	2/49 (4%)	0/49 (0%)
Adjusted rates[b]	8.1%	0.0%	4.3%	0.0%
Terminal rates[c]	3/37 (8%)	0/40 (0%)	2/46 (4%)	0/38 (0%)
First incidence (days)	739 (T)	–[e]	739 (T)	–
Life table tests[d]	P=0.175N	P=0.108N	P=0.401N	P=0.116N
Logistic regression tests[d]	P=0.175N	P=0.108N	P=0.401N	P=0.116N
Cochran-Armitage test[d]	P=0.178N			
Fisher exact test[d]		P=0.117N	P=0.500N	P=0.121N
Liver: Hemangiosarcoma				
Overall rates	1/49 (2%)	3/50 (6%)	0/49 (0%)	1/49 (2%)
Adjusted rates	2.2%	7.5%	0.0%	2.6%
Terminal rates	0/37 (0%)	3/40 (8%)	0/46 (0%)	1/38 (3%)
First incidence (days)	580	739 (T)	–	739 (T)
Life table tests	P=0.465N	P=0.334	P=0.487N	P=0.758
Logistic regression tests	P=0.457N	P=0.307	P=0.616N	P=0.763N
Cochran-Armitage test	P=0.457N			
Fisher exact test		P=0.316	P=0.500N	P=0.753N
Liver: Hepatocellular Adenoma				
Overall rates	7/49 (14%)	12/50 (24%)	17/49 (35%)	41/49 (84%)
Adjusted rates	18.4%	30.0%	36.1%	91.1%
Terminal rates	6/37 (16%)	12/40 (30%)	16/46 (35%)	34/38 (89%)
First incidence (days)	701	739 (T)	676	541
Life table tests	P<0.001	P=0.198	P=0.066	P<0.001
Logistic regression tests	P<0.001	P=0.185	P=0.041	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.166	P=0.017	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rates	5/49 (10%)	5/50 (10%)	6/49 (12%)	12/49 (24%)
Adjusted rates	13.5%	12.0%	13.0%	30.6%
Terminal rates	5/37 (14%)	4/40 (10%)	6/46 (13%)	11/38 (29%)
First incidence (days)	739 (T)	648	739 (T)	689
Life table tests	P=0.012	P=0.578N	P=0.603N	P=0.061
Logistic regression tests	P=0.012	P=0.586N	P=0.603N	P=0.061
Cochran-Armitage test	P=0.014			
Fisher exact test		P=0.617N	P=0.500	P=0.054
Liver: Hepatoblastoma or Hepatocellular Carcinoma				
Overall rates	5/49 (10%)	5/50 (10%)	6/49 (12%)	13/49 (27%)
Adjusted rates	13.5%	12.0%	13.0%	33.2%
Terminal rates	5/37 (14%)	4/40 (10%)	6/46 (13%)	12/38 (32%)
First incidence (days)	739 (T)	648	739 (T)	689
Life table tests	P=0.005	P=0.578N	P=0.603N	P=0.038
Logistic regression tests	P=0.005	P=0.586N	P=0.603N	P=0.038
Cochran-Armitage test	P=0.006			
Fisher exact test		P=0.617N	P=0.500	P=0.033

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rates	10/49 (20%)	15/50 (30%)	21/49 (43%)	45/49 (92%)
Adjusted rates	26.3%	36.4%	44.7%	100.0%
Terminal rates	9/37 (24%)	14/40 (35%)	20/46 (43%)	38/38 (100%)
First incidence (days)	701	648	676	541
Life table tests	P<0.001	P=0.239	P=0.072	P<0.001
Logistic regression tests	P<0.001	P=0.226	P=0.045	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.193	P=0.014	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	5/49 (10%)	7/50 (14%)	4/49 (8%)	1/49 (2%)
Adjusted rates	13.5%	17.5%	8.7%	2.6%
Terminal rates	5/37 (14%)	7/40 (18%)	4/46 (9%)	1/38 (3%)
First incidence (days)	739 (T)	739 (T)	739 (T)	739 (T)
Life table tests	P=0.038N	P=0.434	P=0.365N	P=0.096N
Logistic regression tests	P=0.038N	P=0.434	P=0.365N	P=0.096N
Cochran-Armitage test	P=0.041N			
Fisher exact test		P=0.394	P=0.500N	P=0.102N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	2/48 (4%)	6/50 (12%)	2/49 (4%)	0/45 (0%)
Adjusted rates	5.6%	15.0%	4.3%	0.0%
Terminal rates	2/36 (6%)	6/40 (15%)	2/46 (4%)	0/35 (0%)
First incidence (days)	739 (T)	739 (T)	739 (T)	–
Life table tests	P=0.056N	P=0.169	P=0.604N	P=0.244N
Logistic regression tests	P=0.056N	P=0.169	P=0.604N	P=0.244N
Cochran-Armitage test	P=0.061N			
Fisher exact test		P=0.148	P=0.684N	P=0.264N
All Organs: Hemangiosarcoma				
Overall rates	1/49 (2%)	6/50 (12%)	0/49 (0%)	2/49 (4%)
Adjusted rates	2.2%	14.0%	0.0%	5.3%
Terminal rates	0/37 (0%)	4/40 (10%)	0/46 (0%)	2/38 (5%)
First incidence (days)	580	522	–	739 (T)
Life table tests	P=0.442N	P=0.077	P=0.487N	P=0.501
Logistic regression tests	P=0.425N	P=0.046	P=0.616N	P=0.500
Cochran-Armitage test	P=0.434N			
Fisher exact test		P=0.059	P=0.500N	P=0.500
All Organs: Hemangioma or Hemangiosarcoma				
Overall rates	2/49 (4%)	6/50 (12%)	1/49 (2%)	4/49 (8%)
Adjusted rates	4.6%	14.0%	2.2%	10.5%
Terminal rates	0/37 (0%)	4/40 (10%)	1/46 (2%)	4/38 (11%)
First incidence (days)	580	522	739 (T)	739 (T)
Life table tests	P=0.485	P=0.169	P=0.452N	P=0.346
Logistic regression tests	P=0.502	P=0.106	P=0.594N	P=0.337
Cochran-Armitage test	P=0.495			
Fisher exact test		P=0.141	P=0.500N	P=0.339

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)				
Overall rates	7/49 (14%)	11/50 (22%)	8/49 (16%)	5/49 (10%)
Adjusted rates	17.5%	24.5%	16.6%	12.7%
Terminal rates	5/37 (14%)	7/40 (18%)	6/46 (13%)	4/38 (11%)
First incidence (days)	518	590	532	694
Life table tests	P=0.172N	P=0.289	P=0.559N	P=0.362N
Logistic regression tests	P=0.162N	P=0.221	P=0.467	P=0.376N
Cochran-Armitage test	P=0.163N			
Fisher exact test		P=0.232	P=0.500	P=0.380N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rates	7/49 (14%)	12/50 (24%)	8/49 (16%)	5/49 (10%)
Adjusted rates	17.5%	26.0%	16.6%	12.7%
Terminal rates	5/37 (14%)	7/40 (18%)	6/46 (13%)	4/38 (11%)
First incidence (days)	518	554	532	694
Life table tests	P=0.148N	P=0.221	P=0.559N	P=0.362N
Logistic regression tests	P=0.133N	P=0.142	P=0.467	P=0.376N
Cochran-Armitage test	P=0.137N			
Fisher exact test		P=0.166	P=0.500	P=0.380N
All Organs: Benign Neoplasms				
Overall rates	20/49 (41%)	21/50 (42%)	26/49 (53%)	42/49 (86%)
Adjusted rates	49.7%	52.5%	55.3%	91.3%
Terminal rates	17/37 (46%)	21/40 (53%)	25/46 (54%)	34/38 (89%)
First incidence (days)	184	739 (T)	676	108
Life table tests	P<0.001	P=0.536N	P=0.485	P<0.001
Logistic regression tests	P<0.001	P=0.550N	P=0.221	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.534	P=0.156	P<0.001
All Organs: Malignant Neoplasms				
Overall rates	14/49 (29%)	24/50 (48%)	17/49 (35%)	24/49 (49%)
Adjusted rates	33.6%	48.9%	34.7%	55.6%
Terminal rates	10/37 (27%)	15/40 (38%)	14/46 (30%)	19/38 (50%)
First incidence (days)	518	522	532	108
Life table tests	P=0.102	P=0.086	P=0.576N	P=0.052
Logistic regression tests	P=0.090	P=0.033	P=0.339	P=0.031
Cochran-Armitage test	P=0.090			
Fisher exact test		P=0.037	P=0.332	P=0.031
All Organs: Benign or Malignant Neoplasms				
Overall rates	29/49 (59%)	35/50 (70%)	34/49 (69%)	46/49 (94%)
Adjusted rates	65.6%	71.4%	69.4%	100.0%
Terminal rates	22/37 (59%)	26/40 (65%)	31/46 (67%)	38/38 (100%)
First incidence (days)	184	522	532	108
Life table tests	P<0.001	P=0.335	P=0.472N	P=0.002
Logistic regression tests	P<0.001	P=0.185	P=0.232	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.180	P=0.200	P<0.001

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, gallbladder, heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, salivary gland, spleen, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE D4a
Historical Incidence of Hepatocellular Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	7/49	5/49	10/49
dl-Amphetamine Sulfate	5/50	0/50	5/50
Overall Historical Incidence			
Total	159/1,363 (11.7%)	80/1,363 (5.9%)	223/1,363 (16.4%)
Standard deviation	8.3%	5.5%	10.7%
Range	0%-33%	0%-20%	3%-42%

[a] Data as of 20 August 1992

TABLE D4b
Historical Incidence of Small Intestine Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma or Adenomatous Polyp	Carcinoma	Adenoma, Adenomatous Polyp, or Carcinoma
Historical Incidence at Microbiological Associates, Inc.			
C.I. Direct Blue 218	0/49	0/49	0/49
dl-Amphetamine Sulfate	0/50	0/50	0/50
Overall Historical Incidence			
Total	2/1,371 (0.1%)	8/1,371 (0.6%)	10/1,371 (0.7%)
Standard deviation	1.0%	1.3%	1.3%
Range	0%-2%	0%-6%	0%-6%

[a] Data as of 20 August 1992

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Accidental deaths				1
Moribund	8	8	1	5
Natural deaths	4	2	2	5
Survivors				
Terminal sacrifice	37	40	46	38
Missing	1		1	1
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Intestine small, jejunum	(10)	(1)		(10)
Hyperplasia, lymphoid		1 (100%)		
Liver	(10)	(10)	(10)	(10)
Basophilic focus				1 (10%)
Clear cell focus			2 (20%)	1 (10%)
Eosinophilic focus				6 (60%)
Infiltration cellular, lymphocyte	1 (10%)			
Inflammation, chronic	1 (10%)			
Vocuolization, cytoplasmic	2 (20%)			1 (10%)
Mesentery		(1)		
Fat, infiltration cellular, lymphocytic		1 (100%)		
Pancreas	(10)			(10)
Duct, dilatation				1 (10%)
Infiltration cellular, lymphocyte	2 (20%)			
Salivary gland	(10)			(10)
Infiltration cell, lymphocytic	5 (50%)			5 (50%)
Stomach	(10)	(1)		(10)
Glandular, hyperplasia		1 (100%)		
Cardiovascular System				
None				
Endocrine System				
Thyroid	(10)			(10)
Follicle, dilatation				3 (30%)
General Body System				
None				

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
15-Month Interim Evaluation (continued)				
Genital System				
Ovary	(10)	(1)	(1)	(10)
Cyst	4 (40%)	1 (100%)	1 (100%)	6 (60%)
Uterus	(10)	(4)	(2)	(10)
Dilatation	2 (20%)			
Endometrium, cystic hyperplasia	9 (90%)	4 (100%)	2 (100%)	10 (100%)
Hematopoietic System				
Spleen	(10)	(1)	(10)	
Hyperplasia, lymphoid		1 (100%)		
Lymph Node	(1)			
Renal, hyperplasia, lymphoid	1 (100%)			
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
Brain	(10)			(10)
Mineralization	6 (60%)			7 (70%)
Respiratory System				
Lung	(10)	(1)		(10)
Hemmorrhage				1 (10%)
Infiltration cellular, lymphocyte				1 (10%)
Nose	(10)			(10)
Inflammation, acute	1 (10%)			
Special Senses System				
None				
Urinary System				
Kidney	(10)			(10)
Infiltration cellular, lymphocytic	1 (10%)			
Urinary Bladder	(10)			(10)
Infiltration cellular, lymphocytic	6 (60%)			9 (90%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study				
Alimentary System				
Gallbladder	(47)	(45)	(46)	(42)
Dilatation		1 (2%)	1 (2%)	
Intestine large, cecum	(47)	(50)	(48)	(45)
Hyperplasia, lymphoid		1 (2%)		
Intestine small, duodenum	(46)	(50)	(48)	(46)
Hyperplasia, lymphoid	1 (2%)	1 (2%)		
Intussusception		1 (2%)		
Liver	(49)	(50)	(49)	(49)
Angiectasis, focal	4 (8%)	3 (6%)		
Basophilic focus	4 (8%)	2 (4%)	6 (12%)	7 (14%)
Clear cell focus	2 (4%)	2 (4%)	1 (2%)	12 (24%)
Cyst	1 (2%)			
Eosinophilic focus	11 (22%)	7 (14%)	11 (22%)	21 (43%)
Fatty change, focal	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, focal, lymphocyte		2 (4%)	1 (2%)	
Infiltration cellular, histiocyte	1 (2%)			
Inflammation, chronic, focal	1 (2%)			
Mixed cell focus	1 (2%)	1 (2%)		
Necrosis, focal	2 (4%)	1 (2%)		2 (4%)
Centrilobular, cytomegaly		1 (2%)		
Mesentery	(6)	(5)	(3)	(5)
Fat, necrosis, focal	6 (100%)	2 (40%)	3 (100%)	3 (60%)
Pancreas	(49)	(50)	(48)	(49)
Accessory spleen	1 (2%)	1 (2%)		
Fibrosis, focal				1 (2%)
Infiltration cellular, focal, lymphocyte	1 (2%)			
Inflammation, chronic active, focal	2 (4%)	1 (2%)		
Acinar cell, atrophy				2 (4%)
Acinar cell, hypertrophy, focal	1 (2%)			
Duct, dilatation		2 (4%)		2 (4%)
Salivary glands	(49)	(50)	(49)	(48)
Arteriole, inflammation, chronic, multifocal	1 (2%)			
Stomach, forestomach	(49)	(50)	(48)	(49)
Hyperplasia, focal			1 (2%)	2 (4%)
Ulcer, focal		2 (4%)	2 (4%)	1 (2%)
Stomach, glandular	(49)	(50)	(48)	(49)
Inflammation, acute, focal		1 (2%)		
Cardiovascular System				
Heart	(49)	(50)	(49)	(49)
Cardiomyopathy		1 (2%)		
Arteriole, inflammation, chronic, focal	1 (2%)			
Endocrine System				
Adrenal cortex	(49)	(49)	(48)	(48)
Cyst	1 (2%)			3 (6%)
Cytoplasmic alteration, focal		1 (2%)	4 (8%)	1 (2%)
Degeneration, focal			1 (2%)	1 (2%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Endocrine System (continued)				
Adrenal cortex (continued)				
Hemorrhage, focal			7 (15%)	1 (2%)
Hyperplasia, focal	1 (2%)			
Hypertrophy, focal			1 (2%)	1 (2%)
Vacuolization cytoplasmic, focal				1 (2%)
Adrenal medulla	(49)	(49)	(48)	(47)
Congestion	1 (2%)			
Degeneration, focal				1 (2%)
Hyperplasia, focal		1 (2%)	1 (2%)	
Islets, pancreatic	(49)	(50)	(48)	(49)
Hyperplasia, focal	1 (2%)			2 (4%)
Parathyroid gland	(33)	(34)	(40)	(40)
Infiltration cellular, focal, lymphocyte		1 (3%)		
Pituitary gland	(48)	(50)	(49)	(45)
Pars distalis, angiectasis, focal	2 (4%)			
Pars distalis, cyst	1 (2%)	1 (2%)		1 (2%)
Pars distalis, hyperplasia, focal	6 (13%)	11 (22%)	7 (14%)	3 (7%)
Thyroid gland	(49)	(50)	(49)	(49)
Infiltration cellular, focal, lymphocyte	2 (4%)			
Inflammation, chronic active, focal			1 (2%)	
Arteriole, inflammation, chronic, focal	1 (2%)			
C-cell, hyperplasia, focal		1 (2%)		1 (2%)
Follicular cell, hyperplasia, focal	11 (22%)	9 (18%)	7 (14%)	6 (12%)
Follicular cell, hypertrophy, focal	1 (2%)			
Follicle, dilatation, focal	2 (4%)		1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(1)			(1)
Inflammation, chronic active	1 (100%)			1 (100%)
Duct, dilatation				1 (100%)
Ovary	(47)	(50)	(48)	(49)
Abscess	2 (4%)			
Angiectasis			1 (2%)	3 (6%)
Cyst	12 (26%)	17 (34%)	15 (31%)	28 (57%)
Ectopic tissue				1 (2%)
Hematocyst		1 (2%)	3 (6%)	2 (4%)
Hemorrhage, focal	1 (2%)			
Hyperplasia, tubular				1 (2%)
Infiltration cellular, focal, lymphocyte		1 (2%)		1 (2%)
Inflammation, chronic, focal	2 (4%)			
Mineralization, focal		1 (2%)	1 (2%)	
Pigmentation, focal		1 (2%)		
Bilateral, abscess	1 (2%)			
Follicle, hyperplasia, cystic, focal		1 (2%)		

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Genital System (continued)				
Uterus	(49)	(50)	(49)	(49)
Dilatation	13 (27%)	12 (24%)	15 (31%)	9 (18%)
Ectopic tissue, focal			1 (2%)	
Inflammation, acute	1 (2%)			
Inflammation, chronic active	2 (4%)			
Thrombosis, focal		1 (2%)		
Endometrium, hyperplasia, cystic	31 (63%)	42 (84%)	38 (78%)	31 (63%)
Hematopoietic System				
Bone marrow	(49)	(50)	(49)	(49)
Angiectasis, focal				1 (2%)
Congestion	1 (2%)			
Hyperplasia	5 (10%)			2 (4%)
Hyperplasia, lymphoid	1 (2%)			
Lymph node	(9)	(10)	(8)	(7)
Iliac, hyperplasia, lymphoid	1 (11%)			
Iliac, hyperplasia, plasma cell	1 (11%)			1 (14%)
Lumbar, hyperplasia, lymphoid	3 (33%)	1 (10%)	1 (13%)	
Lumbar, hyperplasia, plasma cell	1 (11%)			
Mediastinal, hyperplasia, lymphoid	1 (11%)			1 (14%)
Mediastinal, hyperplasia, plasma cell	1 (11%)			
Renal, hyperplasia, lymphoid	2 (22%)			
Renal, hyperplasia, plasma cell	2 (22%)			1 (14%)
Lymph node, mandibular	(49)	(48)	(48)	(47)
Congestion		1 (2%)		
Cyst	2 (4%)	3 (6%)	2 (4%)	3 (6%)
Edema		1 (2%)		
Hemorrhage	1 (2%)		1 (2%)	
Hyperplasia, lymphoid	3 (6%)	7 (15%)	4 (8%)	2 (4%)
Hyperplasia, plasma cell	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, mast cell		1 (2%)		
Inflammation, acute	1 (2%)			
Lymph node, mesenteric	(46)	(48)	(47)	(46)
Angiectasis, focal		1 (2%)		
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Hyperplasia, lymphoid	5 (11%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, plasma cell				1 (2%)
Necrosis	1 (2%)			1 (2%)
Spleen	(48)	(50)	(49)	(49)
Amyloid deposition, focal			1 (2%)	
Depletion lymphoid	1 (2%)			1 (2%)
Hematopoietic cell proliferation	10 (21%)	9 (18%)	3 (6%)	6 (12%)
Hyperplasia, lymphoid	6 (13%)	5 (10%)	10 (20%)	5 (10%)
Thymus	(46)	(48)	(48)	(46)
Atrophy				1 (2%)
Cyst	1 (2%)	2 (4%)		1 (2%)
Depletion lymphoid	1 (2%)			1 (2%)
Hemorrhage, focal		1 (2%)		
Hyperplasia, lymphoid	1 (2%)	3 (6%)	2 (4%)	
Necrosis	1 (2%)	1 (2%)		2 (4%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Integumentary System				
Skin	(49)	(50)	(49)	(49)
Acanthosis, focal		1 (2%)		
Inflammation, acute, focal	1 (2%)		1 (2%)	
Inflammation, chronic active, focal	1 (2%)	4 (8%)	1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(49)	(49)
Fibrous osteodystrophy		1 (2%)		
Intervertebral disc, dysplasia, focal		1 (2%)		
Nervous System				
Brain	(49)	(50)	(49)	(49)
Hemorrhage, focal		1 (2%)		
Hydrocephalus		1 (2%)		
Mineralization, focal	29 (59%)	45 (90%)	34 (69%)	38 (78%)
Necrosis, focal	1 (2%)			
Artery, inflammation, chronic, focal	1 (2%)			
Spinal cord		(1)		
Infiltration cellular, focal, histiocyte		1 (100%)		
Respiratory System				
Lung	(49)	(50)	(49)	(49)
Congestion	1 (2%)		1 (2%)	1 (2%)
Hemorrhage, focal	1 (2%)	1 (2%)	1 (2%)	
Hemorrhage, multifocal				1 (2%)
Infiltration cellular, focal, lymphocyte	1 (2%)			
Infiltration cellular, focal, histiocyte		2 (4%)		
Alveolar epithelium, hyperplasia, focal				2 (4%)
Arteriole, hypertrophy, focal			1 (2%)	
Interstitium, inflammation, focal		1 (2%)	3 (6%)	
Nose	(49)	(50)	(49)	(49)
Congestion			1 (2%)	
Inflammation, acute, focal		2 (4%)		
Special Senses System				
Ear	(2)	(1)		
Arteriole, inflammation, chronic, focal	1 (50%)			
Pinna, inflammation, chronic active, focal		1 (100%)		
Eye	(2)	(1)	(1)	
Cornea, inflammation, chronic	2 (100%)			
Lens, cataract		1 (100%)	1 (100%)	
Zymbal's gland	(38)	(38)	(37)	(32)
Inflammation, acute, focal	1 (3%)		1 (3%)	
Inflammation, chronic active			1 (3%)	
Duct, dilatation	1 (3%)	1 (3%)		

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
2-Year Study (continued)				
Urinary System (continued)				
Kidney	(49)	(50)	(49)	(49)
Infarct, chronic, focal			1 (2%)	1 (2%)
Infiltration cellular, focal, lymphocyte	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Metaplasia, focal, osseous	2 (4%)	1 (2%)		2 (4%)
Nephropathy	4 (8%)	3 (6%)	5 (10%)	3 (6%)
Artery, inflammation, chronic, focal	1 (2%)			
Glomerulus, inflammation, chronic	1 (2%)	1 (2%)		
Renal tubule, dilatation, focal			2 (4%)	
Urinary bladder	(48)	(50)	(48)	(49)
Dilatation		1 (2%)	1 (2%)	
Inflammation, acute	1 (2%)			
Inflammation, chronic active, focal				2 (4%)
Arteriole, inflammation, chronic, focal	1 (2%)			

[a] Number of animals examined microscopically at site and number of animals with lesion

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Mortelmans *et al.* (1986). C.I. Direct Blue 218 was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of C.I. Direct Blue 218. In the absence of toxicity, 10,000 μg/plate was selected as the high dose. All positive trials were repeated under the conditions that elicited the positive response.

In this assay, a positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or was not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to the judged positive or weakly positive.

Salmonella typhimurium Assay With Reductive Metabolism Protocol

Details of the experimental technique are presented in Reid *et al.* (1983, 1984) and Prival and Mitchell (1982). Briefly, uncoded aliquots were obtained from Radian Corporation. Overnight Difco nutrient broth cultures of *Salmonella typhimurium* TA1538 were used. The S9 fraction was from Aroclor-induced male Fischer rat liver or non-induced female hamster liver. In the bacterial reduction system, C.I. Direct Blue 218 was reduced overnight by incubation in brain/heart infusion broth with a washed suspension of rat cecal bacteria. Extracts of the reduction mixtures were dissolved in dimethylsulfoxide (DMSO) and combined with TA1538 and rat liver S9 mix (metabolic activation enzymes and cofactors). This mixture was incubated with shaking for 20 minutes at 37° C. Top agar was then added, and the mixtures were plated onto minimal glucose agar plates. Incubation was continued for an additional 72 hours. For the flavin mononucleotide (FMN) reduction system, FMN was added to the DMSO solution containing the hamster liver S9 mix, TA1538, and C.I. Direct Blue 218 and incubated for 20 minutes at 37° C. The mixtures were then plated and incubated as described for the bacterial reduction system.

Each test consisted of triplicate plates of the negative control and three doses of C.I. Direct Blue 218. The positive control, 3,3′-dimethoxybenzidine, was tested at the same molar concentrations as C.I. Direct Blue 218 for each test condition.

Chinese Hamster Ovary Cell Cytogenetics Test Protocols

Testing was performed as reported by Galloway *et al.* (1987). C.I. Direct Blue 218 was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least five doses of C.I. Direct

Blue 218; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with C.I. Direct Blue 218 in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing C.I. Direct Blue 218 was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with C.I. Direct Blue 218, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no C.I. Direct Blue 218, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with C.I. Direct Blue 218 for 10 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with C.I. Direct Blue 218 and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. Fifty-five to 100 first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P\leq0.05$) difference for one dose point and a significant trend ($P\leq0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987).

Drosophila melanogaster Test Protocol

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Woodruff *et al.* (1985). C.I. Direct Blue 218 was supplied as a coded aliquot by Radian Corporation. It was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, C.I. Direct Blue 218 was retested by injection into adult males.

To administer a chemical by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 μL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector that automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of C.I. Direct Blue 218 at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of C.I. Direct Blue 218 in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of C.I. Direct Blue 218 dissolved in saline/peanut oil and allowed to recover for 24 hours. A concurrent saline/peanut oil control group was also included. Treated males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier post-meiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. After 17 days, presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls, using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater than 0.10%, or if the P value was greater than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15%, or (b) the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

Results

C.I. Direct Blue 218 (33 to 10,000 μg/plate) did not induce mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the standard NTP assay, which used a preincubation protocol, with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 oxidative enzymes (Table E1; Mortelmans *et al.,* 1986). C.I. Direct Blue 218 was also tested in a modified *Salmonella* test protocol which employed reductive metabolism supplied by flavin mononucleotide or rat cecal bacteria, followed by oxidative metabolism; results of this test, using strain TA1538, were also negative (Table E2; Reid *et al.,* 1984).

In cytogenetic tests with CHO cells, C.I. Direct Blue 218 induced a small but significant increase in SCEs at the highest dose tested (200 μg/mL) in the second of two trials conducted without S9 (Table E3); no increase in SCEs was observed in either of two trials conducted with S9. No increase in chromosomal aberrations was seen in CHO cells treated with C.I. Direct Blue 218, at concentrations up to 500 μg/mL, with and without S9 (Table E4).

No increase in the frequency of sex-linked recessive lethal mutations was observed in the offspring of male *Drosophila melanogaster* administered C.I. Direct Blue 218 by feeding (10,000 ppm) or by injection (1,000 ppm) (Table E5; Woodruff *et al.,* 1985).

In summary, genetic toxicity tests with C.I. Direct Blue 218 indicate that the chemical is not genotoxic.

TABLE E1
Mutagenicity of C.I. Direct Blue 218 in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	97 ± 2.3	125 ± 3.5	101 ± 5.9	104 ± 3.0	108 ± 15.1	97 ± 4.2
	100	73 ± 3.7	39 ± 39.3	130 ± 1.2	95 ± 5.0	110 ± 8.2	92 ± 5.2
	333	82 ± 13.9	133 ± 16.0	104 ± 2.0	96 ± 3.8	124 ± 3.5	88 ± 1.0
	1,000	94 ± 5.4	125 ± 5.4	100 ± 3.3	84 ± 4.6	106 ± 8.2	91 ± 3.9
	3,333	91 ± 2.3	125 ± 13.1	112 ± 9.0	102 ± 8.4	105 ± 6.9	85 ± 1.5
	10,000	66 ± 2.6	136 ± 4.9	85 ± 11.0	69 ± 0.3	105 ± 9.0	67 ± 4.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		439 ± 41.8	1,475 ± 37.4	1,678 ± 99.2	2,285 ± 33.2	1,642 ± 41.2	2,205 ± 35.2
TA1535	0	3 ± 0.9	17 ± 6.0	4 ± 0.6	5 ± 1.5	9 ± 1.8	7 ± 3.4
	33				6 ± 0.9		5 ± 0.9
	100	6 ± 1.2	20 ± 2.9	5 ± 1.5	2 ± 0.7	6 ± 0.3	5 ± 1.8
	333	2 ± 0.6	21 ± 3.2	4 ± 1.2	2 ± 0.6	4 ± 0.9	3 ± 1.2
	1,000	5 ± 1.7	24 ± 1.2	11 ± 1.0	1 ± 0.6	0 ± 0.0	2 ± 0.9
	3,333	4 ± 1.5	20 ± 3.3	1 ± 1.0	0 ± 0.3	1 ± 0.3	0 ± 0.3
	10,000	3 ± 0.7	19 ± 0.3	1 ± 0.7		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		126 ± 11.1	1,224 ± 8.5	197 ± 38.9	149 ± 30.0	102 ± 16.2	36 ± 8.4
TA1537	0	8 ± 1.2	9 ± 0.3	4 ± 1.2	5 ± 1.5	6 ± 1.5	5 ± 1.8
	100	3 ± 0.9	7 ± 0.5	6 ± 0.9	5 ± 1.0	8 ± 1.5	4 ± 0.6
	333	6 ± 1.7	12 ± 2.2	7 ± 1.5	4 ± 1.5	6 ± 1.5	2 ± 1.2
	1,000	5 ± 0.3	9 ± 0.0	7 ± 0.9	3 ± 0.0	3 ± 0.9	2 ± 0.3
	3,333	4 ± 0.7	10 ± 0.9	6 ± 0.9	1 ± 0.6	4 ± 1.2	2 ± 0.3
	10,000	1 ± 0.3	7 ± 1.0	4 ± 0.3	2 ± 1.2	3 ± 0.3	1 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		116 ± 13.4	168 ± 37.8	120 ± 8.0	153 ± 41.4	204 ± 29.4	34 ± 8.6
TA98	0	20 ± 0.6	28 ± 2.0	22 ± 3.8	21 ± 1.2	29 ± 0.9	22 ± 2.0
	100	17 ± 1.2	20 ± 3.1	27 ± 1.5	20 ± 1.0	24 ± 1.8	15 ± 2.8
	333	13 ± 2.6	21 ± 4.3	27 ± 6.4	17 ± 0.6	30 ± 4.7	19 ± 1.5
	1,000	15 ± 0.9	19 ± 1.3	26 ± 2.2	20 ± 0.7	18 ± 0.3	17 ± 0.6
	3,333	15 ± 2.4	19 ± 1.5	12 ± 1.2	17 ± 0.6	19 ± 5.8	15 ± 1.5
	10,000	13 ± 1.2	24 ± 3.8	17 ± 1.5	8 ± 1.5	16 ± 5.5	9 ± 0.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		170 ± 15.4	199 ± 17.6	2,076 ± 87.7	1,985 ± 18.2	942 ± 83.8	2,081 ± 44.7

[a] Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Mortelmans *et al.* (1986). 0 μg/plate is the solvent control.

[b] Revertants are presented as mean ± the standard error from three plates.

[c] 2-Aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE E2
Mutagenicity of C.I. Direct Blue 218 in *Salmonella typhimurium* Strain TA1538 in Rat Cecal Bacterial and Flavin Mononucleotide (FMN) Reduction Systems

Dose (μM)	Reductive System/Oxidative System (revertants/plate)[a]		
	Bacterial/rat S9	None/rat S9	FMN/hamster S9
0.00	43	35	33
0.25	40 (790)[b]	14 (843)	27 (235)
0.50	47 (1,073)	16 (1,203)	21 (316)

[a] Revertants are the average from three plates.
[b] The number of revertants obtained with the positive control, 3,3′-dimethoxybenzidine at equimolar concentrations, is given in parentheses after the values obtained for C.I. Direct Blue 218.

TABLE E3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by C.I. Direct Blue 218[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[b]
–S9								
Trial 1								
Summary: Negative								
Medium		50	1,039	398	0.38	8.0	26.0	
Mitomycin-C	0.01	50	1,037	2,209	2.13	44.2	26.0	456.10
C.I. Direct Blue 218								
	0.16	50	1,035	424	0.40	8.5	26.0	6.94
	5.00	50	1,033	440	0.42	8.8	26.0	11.19
	16.00	50	1,038	461	0.44	9.2	26.0	15.94
	50.00	50	1,043	426	0.40	8.5	26.0	6.62
	160.00	50	1,038	457	0.44	9.1	26.0	14.93
								P=0.025[c]
Trial 2								
Summary: Weak positive								
Medium		50	1,034	414	0.40	8.3	26.0	
Mitomycin-C	0.005	50	1,047	1,430	1.36	28.6	26.0	241.13
	0.010	50	1,046	1,854	1.77	37.1	26.0	342.69
C.I. Direct Blue 218								
	25	50	1,036	426	0.41	8.5	26.0	2.70
	50	50	1,039	414	0.39	8.3	26.0	–0.48
	75	50	1,038	404	0.38	8.1	26.0	–2.79
	100	50	1,043	488	0.46	9.8	26.0	16.86
	200	50	1,034	561	0.54	11.2	26.0	35.51*
	300	0						
								P<0.001

TABLE E3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by C.I. Direct Blue 218
(continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9								
Trial 1								
Summary: Negative								
Medium								
		50	1,036	502	0.48	10.0	26.0	
Cyclophosphamide								
	1.50	50	1,042	1,723	1.65	34.5	26.0	241.25
C.I. Direct Blue 218								
	1.6	50	1,042	426	0.40	8.5	26.0	−15.63
	5	50	1,046	434	0.41	8.7	26.0	−14.37
	16	50	1,046	401	0.38	8.0	26.0	−20.88
	50	50	1,042	467	0.44	9.3	26.0	−7.51
	160	50	1,035	509	0.49	10.2	26.0	1.49
								P=0.237
Trial 2								
Summary: Negative								
Medium								
		50	1,029	406	0.39	8.1	26.0	
Cyclophosphamide								
	1.50	50	1,017	1,763	1.73	35.3	26.0	339.37
C.I. Direct Blue 218								
	25	50	1,030	412	0.40	8.2	26.0	1.38
	50	50	1,029	435	0.42	8.7	26.0	7.14
	100	50	1,031	438	0.42	8.8	26.0	7.67
	150	50	1,027	465	0.45	9.3	26.0	14.76
	200	50	1,007	454	0.45	9.1	26.0	14.27
								P=0.005

* Positive (≥ 20% increase over solvent control)
[a] Study performed at Environmental Health Research & Testing, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1987).
[b] SCEs/chromosome of culture exposed to C.I. Direct Blue 218 relative to those of culture exposed to solvent.
[c] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE E4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by C.I. Direct Blue 218[a]

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells w/Abs
Trial 1 - Harvest time: 12.0 hours Summary: Negative					**Trial 1** - Harvest time: 12.0 hours Summary: Negative				
Distilled water					Distilled water				
	100	0	0.00	0.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.50	100	52	0.52	31.0	25.0	100	48	0.48	31.0
C.I. Direct Blue 218					C.I. Direct Blue 218				
5	100	0	0.00	0.0	1.6	100	0	0.00	0.0
16	100	0	0.00	0.0	5.0	100	0	0.00	0.0
50	100	0	0.00	0.0	16.0	100	0	0.00	0.0
160	100	0	0.00	0.0	50.0	100	0	0.00	0.0
500	100	0	0.00	0.0	160.0	100	1	0.01	1.0
					500.0	0			
				P=0.500[b]					P=0.071
Trial 2 - Harvest time: 12.0 hours Summary: Negative					**Trial 2** - Harvest time: 12.0 hours Summary: Negative				
Distilled water					Distilled water				
	100	0	0.00	0.0		100	3	0.03	3.0
Mitomycin-C					Cyclophosphamide				
0.50	100	47	0.47	34.0	25.0	100	76	0.76	47.0
C.I. Direct Blue 218					C.I. Direct Blue 218				
100	100	0	0.00	0.0	50	100	1	0.01	1.0
200	100	0	0.00	0.0	100	100	2	0.02	2.0
300	100	0	0.00	0.0	200	100	3	0.03	3.0
400	100	0	0.00	0.0	300	100	5	0.05	5.0
500	100	0	0.00	0.0	400	55	1	0.02	
					500	100	1	0.01	1.0
				P=0.500					P=0.413

[a] Study performed at Environmental Health Research & Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1987).

[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

TABLE E5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by C.I. Direct Blue 218[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[b]
Injection	1,000	10	1	2/1,784	1/1,859	5/1,885	8/5,528 (0.14%)
	0			2/1,802	4/1,906	3/1,939	9/5,647 (0.16%)
Feeding	10,000	5	9	0/1,919	0/1,980	1/1,852	1/5,751 (0.02%)
	0			0/1,952	2/1,889	0/1,866	2/5,707 (0.04%)

[a] Study performed at Brown University. A detailed protocol of the sex-linked recessive lethal assay and these data are presented in Woodruff *et al.* (1985). Results were not significant at the 5% level (Margolin *et al.* 1983).
[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials.

APPENDIX F
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	7,000 ppm	15,000 ppm	30,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	237 ± 8	238 ± 9	232 ± 9	230 ± 7	226 ± 7	182 ± 10**
Brain						
Absolute	1.807 ± 0.027	1.827 ± 0.016	1.859 ± 0.016	1.864 ± 0.019	1.846 ± 0.031	1.796 ± 0.025
Relative	7.66 ± 0.14	7.70 ± 0.30	8.05 ± 0.24	8.14 ± 0.27	8.20 ± 0.25	10.00 ± 0.52**
Heart						
Absolute	0.962 ± 0.031	0.913 ± 0.025	0.903 ± 0.042	0.914 ± 0.028	0.881 ± 0.047	0.668 ± 0.036**
Relative	4.07 ± 0.06	3.84 ± 0.08	3.89 ± 0.08	3.98 ± 0.04	3.92 ± 0.25	3.68 ± 0.08*
R. Kidney						
Absolute	1.047 ± 0.040	1.022 ± 0.055	1.097 ± 0.050	1.120 ± 0.042	1.090 ± 0.035	0.872 ± 0.046*
Relative	4.42 ± 0.05	4.28 ± 0.08	4.72 ± 0.08	4.87 ± 0.14*	4.83 ± 0.09*	4.81 ± 0.15*
Liver						
Absolute	10.263 ± 0.678	9.621 ± 0.595	10.292 ± 0.830	10.201 ± 0.174	9.918 ± 0.393	6.717 ± 0.523**
Relative	43.33 ± 2.19	40.38 ± 2.20	44.12 ± 2.29	44.59 ± 1.90	44.11 ± 2.30	36.91 ± 1.76
Lungs						
Absolute	1.283 ± 0.089	1.309 ± 0.033	1.260 ± 0.056	1.285 ± 0.031	1.282 ± 0.071	1.099 ± 0.083
Relative	5.43 ± 0.37	5.52 ± 0.24	5.45 ± 0.25	5.60 ± 0.10	5.69 ± 0.29	6.06 ± 0.35
R. Testis						
Absolute	1.326 ± 0.016	1.373 ± 0.051	1.299 ± 0.055	1.336 ± 0.041	1.301 ± 0.028	1.197 ± 0.054
Relative	5.62 ± 0.13	5.76 ± 0.13	5.60 ± 0.10	5.81 ± 0.04	5.77 ± 0.13	6.60 ± 0.10**
Thymus						
Absolute	0.413 ± 0.021	0.389 ± 0.014	0.384 ± 0.028	0.395 ± 0.027	0.386 ± 0.018	0.248 ± 0.022**
Relative	1.75 ± 0.08	1.65 ± 0.11	1.65 ± 0.09	1.73 ± 0.15	1.72 ± 0.10	1.37 ± 0.10*
Female						
Necropsy body wt	149 ± 6	150 ± 5	149 ± 5	148 ± 5	149 ± 5	133 ± 5
Brain						
Absolute	1.718 ± 0.032	1.699 ± 0.014	1.662 ± 0.066	1.700 ± 0.039	1.707 ± 0.016	1.669 ± 0.049
Relative	11.60 ± 0.33	11.41 ± 0.37	11.14 ± 0.16	11.54 ± 0.18	11.52 ± 0.38	12.55 ± 0.28*
Heart						
Absolute	0.652 ± 0.025	0.635 ± 0.017	0.640 ± 0.029	0.643 ± 0.030	0.658 ± 0.021	0.501 ± 0.021**
Relative	4.39 ± 0.10	4.25 ± 0.08	4.29 ± 0.08	4.36 ± 0.13	4.43 ± 0.16	3.76 ± 0.09**
R. Kidney						
Absolute	0.660 ± 0.029	0.685 ± 0.028	0.709 ± 0.026	0.722 ± 0.036	0.762 ± 0.025	0.617 ± 0.011
Relative	4.44 ± 0.09	4.59 ± 0.15	4.76 ± 0.06	4.89 ± 0.18*	5.13 ± 0.19*	4.65 ± 0.16*
Liver						
Absolute	6.023 ± 0.391	6.354 ± 0.348	6.155 ± 0.316	6.065 ± 0.404	6.287 ± 0.390	5.055 ± 0.198
Relative	40.51 ± 2.09	42.48 ± 1.92	41.37 ± 1.93	40.97 ± 1.85	42.34 ± 2.60	37.98 ± 1.11
Lungs						
Absolute	0.938 ± 0.036	0.975 ± 0.068	1.036 ± 0.054	0.989 ± 0.065	1.088 ± 0.130	0.877 ± 0.057
Relative	6.35 ± 0.39	6.53 ± 0.40	6.96 ± 0.30	6.68 ± 0.28	7.30 ± 0.79	6.57 ± 0.25
Thymus						
Absolute	0.330 ± 0.020	0.340 ± 0.020	0.331 ± 0.012	0.331 ± 0.009	0.327 ± 0.028	0.247 ± 0.016**
Relative	2.23 ± 0.15	2.28 ± 0.15	2.23 ± 0.09	2.25 ± 0.07	2.20 ± 0.18	1.85 ± 0.06

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of C.I. Direct Blue 218[a]

	0 ppm	3,000 ppm	10,000 ppm	20,000 ppm
n	10	10	10	10
Male				
Necropsy body wt	318 ± 7	326 ± 4	305 ± 7	232.5 ± 13.7**
Brain				
Absolute	1.854 ± 0.022	1.939 ± 0.015*	1.956 ± 0.024**	1.881 ± 0.019
Relative	5.86 ± 0.14	5.95 ± 0.06	6.43 ± 0.10	8.46 ± 0.72**
Heart				
Absolute	1.134 ± 0.028	1.224 ± 0.025	1.141 ± 0.034	0.955 ± 0.036**
Relative	3.57 ± 0.09	3.75 ± 0.06	3.74 ± 0.07	4.24 ± 0.28**
R. Kidney				
Absolute	1.264 ± 0.030	1.527 ± 0.039**	1.551 ± 0.046**	1.394 ± 0.052**[b]
Relative	3.98 ± 0.09	4.68 ± 0.10*	5.08 ± 0.09**	6.25 ± 0.37**[b]
Liver				
Absolute	10.446 ± 0.364	10.963 ± 0.304	10.351 ± 0.303	8.471 ± 0.274**
Relative	32.86 ± 0.83	33.59 ± 0.74	33.97 ± 0.87	37.65 ± 2.55*
Lungs				
Absolute	1.699 ± 0.095	1.690 ± 0.046	1.672 ± 0.066	1.374 ± 0.054**
Relative	5.36 ± 0.30	5.18 ± 0.13	5.47 ± 0.15	6.12 ± 0.46
R. Testis				
Absolute	1.475 ± 0.027	1.536 ± 0.018	1.470 ± 0.030	0.980 ± 0.140**
Relative	4.65 ± 0.09	4.71 ± 0.06	4.83 ± 0.08	4.20 ± 0.50
Thymus				
Absolute	0.306 ± 0.020	0.298 ± 0.007	0.263 ± 0.016*	0.248 ± 0.010**
Relative	0.96 ± 0.06	0.92 ± 0.02	0.86 ± 0.05	1.11 ± 0.10
Female				
Necropsy body wt	188 ± 4	192 ± 3	179 ± 2*	159 ± 2**
Brain				
Absolute	1.830 ± 0.019	1.826 ± 0.024	1.797 ± 0.020	1.801 ± 0.025
Relative	9.78 ± 0.18	9.54 ± 0.14	10.03 ± 0.12	11.38 ± 0.23**
Heart				
Absolute	0.779 ± 0.022	0.774 ± 0.009	0.719 ± 0.029	0.690 ± 0.018**
Relative	4.15 ± 0.08	4.04 ± 0.05	4.01 ± 0.16	4.36 ± 0.10
R. Kidney				
Absolute	0.805 ± 0.021	0.892 ± 0.027*	0.994 ± 0.036**	0.958 ± 0.018**
Relative	4.29 ± 0.10	4.66 ± 0.13	5.54 ± 0.17**	6.05 ± 0.11**
Liver				
Absolute	6.335 ± 0.167	6.400 ± 0.217	6.494 ± 0.200	5.880 ± 0.111
Relative	33.81 ± 0.91	33.44 ± 1.17	36.19 ± 0.95	37.13 ± 0.79*
Lungs				
Absolute	1.207 ± 0.033	1.380 ± 0.049	1.158 ± 0.025	1.088 ± 0.038*
Relative	6.43 ± 0.16	7.21 ± 0.26*	6.45 ± 0.11	6.88 ± 0.28
Thymus				
Absolute	0.229 ± 0.008	0.262 ± 0.012	0.245 ± 0.011	0.237 ± 0.015
Relative	1.22 ± 0.03	1.37 ± 0.06	1.37 ± 0.06	1.49 ± 0.08**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)
[b] n=9

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male				
n	10	10	10	9
Necropsy body wt	448 ± 13	432 ± 15	468 ± 11	429 ± 6
Brain				
Absolute	2.178 ± 0.013	2.132 ± 0.011*	2.135 ± 0.018*	2.128 ± 0.018*
Relative	4.90 ± 0.15	4.99 ± 0.18	4.58 ± 0.10	4.97 ± 0.06
R. Kidney				
Absolute	1.774 ± 0.042	1.807 ± 0.052	1.847 ± 0.032	1.951 ± 0.049**
Relative	3.98 ± 0.10	4.20 ± 0.07	3.96 ± 0.09	4.56 ± 0.11**
Liver				
Absolute	16.327 ± 0.320	14.921 ± 0.626	15.899 ± 0.296	15.943 ± 0.280
Relative	36.66 ± 0.95	34.57 ± 0.90	34.04 ± 0.65	37.24 ± 0.67
Spleen				
Absolute	0.989 ± 0.070	0.877 ± 0.043	0.951 ± 0.029	1.015 ± 0.093
Relative	2.21 ± 0.14	2.03 ± 0.06	2.03 ± 0.04	2.37 ± 0.23
Female				
n	9	9	10	10
Necropsy body wt	301 ± 9	294 ± 11	285 ± 5	280 ± 8
Brain				
Absolute	1.934 ± 0.014	1.925 ± 0.013	1.915 ± 0.019	1.950 ± 0.022
Relative	6.46 ± 0.17	6.61 ± 0.24	6.74 ± 0.13	7.01 ± 0.16*
R. Kidney				
Absolute	1.043 ± 0.014	1.065 ± 0.038	1.194 ± 0.021**	1.287 ± 0.035**
Relative	3.48 ± 0.10	3.62 ± 0.05	4.19 ± 0.06**	4.61 ± 0.12**
Liver				
Absolute	9.766 ± 0.195	9.664 ± 0.438	9.692 ± 0.283	9.599 ± 0.258
Relative	32.51 ± 0.63	32.77 ± 0.50	33.99 ± 0.63	34.34 ± 0.46*
Spleen				
Absolute	0.604 ± 0.017	0.609 ± 0.024	0.612 ± 0.025	0.601 ± 0.042
Relative	2.01 ± 0.07	2.08 ± 0.09	2.15 ± 0.09	2.16 ± 0.15

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	7,000 ppm	15,000 ppm	30,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	21.6 ± 0.9	22.2 ± 1.0	21.8 ± 0.8	21.5 ± 0.8	21.4 ± 1.3	16.8 ± 0.7**
Brain						
Absolute	0.446 ± 0.009	0.443 ± 0.013	0.455 ± 0.012	0.447 ± 0.011	0.451 ± 0.017	0.439 ± 0.005
Relative	20.78 ± 1.03	20.03 ± 0.53	20.94 ± 0.44	20.78 ± 0.31	21.21 ± 0.81	26.31 ± 1.05**
Heart						
Absolute	0.127 ± 0.007	0.136 ± 0.002	0.139 ± 0.004	0.136 ± 0.005	0.121 ± 0.011	0.106 ± 0.008*
Relative	5.89 ± 0.26	6.18 ± 0.23	6.39 ± 0.13	6.31 ± 0.08	5.61 ± 0.33	6.26 ± 0.23
R. Kidney						
Absolute	0.197 ± 0.009	0.207 ± 0.013	0.212 ± 0.008	0.211 ± 0.016	0.204 ± 0.023	0.146 ± 0.006*
Relative	9.11 ± 0.15	9.31 ± 0.36	9.72 ± 0.12	9.73 ± 0.49	9.43 ± 0.59	8.73 ± 0.19
Liver						
Absolute	0.935 ± 0.054	0.913 ± 0.038	0.947 ± 0.046	0.905 ± 0.046	1.204 ± 0.088**	0.755 ± 0.038
Relative	43.13 ± 0.94	41.17 ± 0.96	43.44 ± 0.97	41.99 ± 0.98	56.09 ± 2.46**	44.97 ± 0.95**
Lungs						
Absolute	0.195 ± 0.011	0.195 ± 0.022	0.204 ± 0.013	0.191 ± 0.017	0.183 ± 0.012	0.145 ± 0.006*
Relative	9.10 ± 0.68	8.71 ± 0.70	9.41 ± 0.78	8.80 ± 0.49	8.55 ± 0.15	8.66 ± 0.33
R. Testis						
Absolute	0.105 ± 0.004	0.106 ± 0.005	0.106 ± 0.005	0.112 ± 0.006	0.098 ± 0.011	0.091 ± 0.004
Relative	4.87 ± 0.19	4.79 ± 0.18	4.85 ± 0.12	5.18 ± 0.14	4.53 ± 0.31	5.44 ± 0.17
Thymus						
Absolute	0.043 ± 0.002	0.030 ± 0.004*	0.036 ± 0.004*	0.034 ± 0.003*	0.033 ± 0.003*	0.013 ± 0.003**
Relative	2.02 ± 0.15	1.36 ± 0.22	1.65 ± 0.22	1.62 ± 0.18	1.51 ± 0.07	0.76 ± 0.17**
Female						
Necropsy body wt	16.9 ± 0.3	17.3 ± 0.4	17.2 ± 0.2	16.8 ± 0.8	16.9 ± 0.3	14.7 ± 0.7**
Brain						
Absolute	0.461 ± 0.010	0.447 ± 0.006	0.448 ± 0.007	0.456 ± 0.012	0.445 ± 0.014	0.438 ± 0.009
Relative	27.22 ± 0.25	25.91 ± 0.45	26.10 ± 0.69	27.27 ± 1.17	26.25 ± 0.49	30.07 ± 1.10**
Heart						
Absolute	0.120 ± 0.005	0.110 ± 0.004	0.109 ± 0.004	0.110 ± 0.008	0.102 ± 0.003*	0.091 ± 0.005**
Relative	7.11 ± 0.25	6.39 ± 0.24*	6.33 ± 0.14*	6.52 ± 0.21*	6.01 ± 0.19**	6.23 ± 0.13**
R. Kidney						
Absolute	0.139 ± 0.006	0.132 ± 0.003	0.143 ± 0.004	0.140 ± 0.012	0.134 ± 0.007	0.113 ± 0.008*
Relative	8.20 ± 0.20	7.65 ± 0.16	8.33 ± 0.25	8.27 ± 0.34	7.88 ± 0.32	7.72 ± 0.21
Liver						
Absolute	0.765 ± 0.028	0.756 ± 0.026	0.787 ± 0.024	0.787 ± 0.024	0.904 ± 0.028	0.777 ± 0.049
Relative	45.16 ± 0.95	43.82 ± 1.35	45.75 ± 0.89	46.93 ± 1.32	53.45 ± 1.73**	53.03 ± 2.61**
Lungs						
Absolute	0.170 ± 0.010	0.172 ± 0.006	0.174 ± 0.005	0.168 ± 0.008	0.170 ± 0.009	0.162 ± 0.021
Relative	10.01 ± 0.45	9.96 ± 0.33	10.11 ± 0.31	10.00 ± 0.12	10.05 ± 0.54	10.98 ± 1.34
Thymus						
Absolute	0.047 ± 0.002	0.053 ± 0.004	0.038 ± 0.005	0.044 ± 0.001	0.032 ± 0.003**	0.015 ± 0.004**
Relative	2.79 ± 0.08	3.09 ± 0.27	2.22 ± 0.30	2.61 ± 0.08	1.88 ± 0.19**	1.01 ± 0.21**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of C.I. Direct Blue 218[a]

	0 ppm	3,000 ppm	10,000 ppm	20,000 ppm
Male				
n	10	9	9	10
Necropsy body wt	27.5 ± 0.8	26.3 ± 0.7	26.1 ± 0.3	22.3 ± 0.9**
Brain				
Absolute	0.472 ± 0.006	0.467 ± 0.010	0.464 ± 0.011	0.471 ± 0.006
Relative	17.28 ± 0.39	17.86 ± 0.65	17.77 ± 0.33	21.40 ± 0.93**
Heart				
Absolute	0.167 ± 0.007	0.163 ± 0.006	0.144 ± 0.005*	0.125 ± 0.007**
Relative	6.10 ± 0.24	6.20 ± 0.26	5.51 ± 0.19	5.54 ± 0.14
R. Kidney				
Absolute	0.254 ± 0.011	0.231 ± 0.007	0.231 ± 0.004	0.213 ± 0.013**
Relative	9.21 ± 0.28	8.80 ± 0.31	8.85 ± 0.10	9.57 ± 0.46
Liver				
Absolute	1.069 ± 0.038	1.005 ± 0.026	1.140 ± 0.031	1.157 ± 0.055
Relative	38.91 ± 0.95	38.31 ± 0.97	43.69 ± 1.18*	52.01 ± 1.85**
Lungs				
Absolute	0.202 ± 0.006	0.195 ± 0.005	0.205 ± 0.006	0.182 ± 0.006*
Relative	7.36 ± 0.19	7.47 ± 0.30	7.86 ± 0.24	8.25 ± 0.32*
R. Testis				
Absolute	0.123 ± 0.004	0.112 ± 0.004	0.120 ± 0.005	0.104 ± 0.007*
Relative	4.48 ± 0.10	4.25 ± 0.13	4.61 ± 0.21	4.58 ± 0.19
Thymus				
Absolute	0.037 ± 0.002	0.033 ± 0.003	0.036 ± 0.002	0.028 ± 0.004
Relative	1.34 ± 0.08	1.24 ± 0.09	1.36 ± 0.06	1.28 ± 0.18
Female				
n	10	10	10	10
Necropsy body wt	22.0 ± 0.7	22.9 ± 0.8	21.2 ± 0.5	19.3 ± 0.3**
Brain				
Absolute	0.469 ± 0.021	0.490 ± 0.008	0.490 ± 0.007	0.487 ± 0.004
Relative	21.50 ± 1.15	21.56 ± 0.55	23.14 ± 0.48	25.27 ± 0.36**
Heart				
Absolute	0.128 ± 0.003	0.122 ± 0.003	0.117 ± 0.004*	0.110 ± 0.002**
Relative	5.83 ± 0.11	5.34 ± 0.13*	5.50 ± 0.13	5.71 ± 0.14
R. Kidney				
Absolute	0.167 ± 0.007	0.177 ± 0.006	0.177 ± 0.005	0.168 ± 0.002
Relative	7.57 ± 0.18	7.73 ± 0.19	8.32 ± 0.13**	8.71 ± 0.15**
Liver				
Absolute	0.908 ± 0.048	0.970 ± 0.032	0.969 ± 0.030	1.005 ± 0.022
Relative	41.10 ± 0.89	42.47 ± 0.82	45.65 ± 1.01**	52.04 ± 0.98**
Lungs				
Absolute	0.185 ± 0.008	0.185 ± 0.007	0.195 ± 0.005	0.166 ± 0.005
Relative	8.41 ± 0.28	8.10 ± 0.29	9.21 ± 0.28	8.60 ± 0.26
Thymus				
Absolute	0.042 ± 0.004	0.041 ± 0.003	0.044 ± 0.002	0.031 ± 0.002*[b]
Relative	1.92 ± 0.16	1.78 ± 0.12	2.06 ± 0.11	1.59 ± 0.11[b]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)
[b] n=9

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluation in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male				
n	9	10	10	10
Necropsy body wt	42.5 ± 0.9	43.6 ± 0.8	41.4 ± 1.1	37.2 ± 1.2**
Brain				
Absolute	0.527 ± 0.005	0.532 ± 0.010	0.538 ± 0.005	0.531 ± 0.006
Relative	12.44 ± 0.27	12.22 ± 0.29	13.11 ± 0.40	14.38 ± 0.43**
R. Kidney				
Absolute	0.419 ± 0.016	0.419 ± 0.010	0.397 ± 0.013	0.407 ± 0.015
Relative	9.84 ± 0.30	9.59 ± 0.16	9.61 ± 0.19	10.95 ± 0.23**
Liver				
Absolute	1.852 ± 0.231	1.730 ± 0.067	1.571 ± 0.062	1.552 ± 0.057
Relative	43.13 ± 4.63	39.62 ± 1.26	37.96 ± 0.86	41.77 ± 1.01
Spleen				
Absolute	0.079 ± 0.009	0.080 ± 0.010	0.077 ± 0.007	0.073 ± 0.004
Relative	1.84 ± 0.20	1.82 ± 0.20	1.88 ± 0.17	1.95 ± 0.08
Female				
n	10	10	10	10
Necropsy body wt	43.33 ± 1.25	41.91 ± 1.52	40.19 ± 0.82	34.44 ± 1.25**
Brain				
Absolute	0.545 ± 0.007	0.542 ± 0.008	0.536 ± 0.008	0.546 ± 0.005
Relative	12.66 ± 0.40	13.08 ± 0.49	13.37 ± 0.29	16.04 ± 0.65**
R. Kidney				
Absolute	0.265 ± 0.003	0.267 ± 0.008	0.261 ± 0.007	0.267 ± 0.008
Relative	6.18 ± 0.20	6.40 ± 0.22	6.51 ± 0.20	7.81 ± 0.25**
Liver				
Absolute	1.575 ± 0.027	1.560 ± 0.029	1.581 ± 0.038	1.623 ± 0.068
Relative	36.53 ± 0.86	37.48 ± 0.91	39.48 ± 1.21	47.55 ± 2.40**
Spleen				
Absolute	0.107 ± 0.004	0.130 ± 0.020	0.121 ± 0.008	0.101 ± 0.003
Relative	2.50 ± 0.12	3.16 ± 0.55	3.03 ± 0.23	2.98 ± 0.15

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

APPENDIX G
HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS

TABLE G1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of C.I. Direct Blue 218[a]

	0 ppm	3,000 ppm	10,000 ppm	20,000 ppm
Male				
n	10	10	10	10
Hematology				
Hematocrit (%)	44.8 ± 0.4	44.7 ± 0.5	42.1 ± 0.5**	41.8 ± 0.5**
Hemoglobin (g/dL)	17.4 ± 0.2	17.4 ± 0.2	16.5 ± 0.2**	16.5 ± 0.2**
Erythrocytes ($10^6/\mu L$)	8.72 ± 0.08	8.77 ± 0.10	8.46 ± 0.13	9.09 ± 0.12
Mean cell volume (fL)	51.4 ± 0.2	50.9 ± 0.1*	49.7 ± 0.2**	46.0 ± 0.3**
Mean cell hemoglobin (pg)	19.9 ± 0.1	19.8 ± 0.1	19.4 ± 0.1**	18.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	38.8 ± 0.2	38.9 ± 0.1	39.2 ± 0.2	39.5 ± 0.1**
Leukocytes ($10^3/\mu L$)	7.20 ± 0.47	7.31 ± 0.47	6.72 ± 0.45	6.43 ± 0.55
Segmented neutrophils ($10^3/\mu L$)	1.49 ± 0.13	1.49 ± 0.08	1.34 ± 0.21	1.23 ± 0.11
Lymphocytes ($10^3/\mu L$)	5.53 ± 0.40	5.59 ± 0.44	5.26 ± 0.33	5.11 ± 0.43
Monocytes ($10^3/\mu L$)	0.15 ± 0.02[b]	0.13 ± 0.02[c]	0.10 ± 0.00[d]	0.14 ± 0.02[e]
Eosinophils ($10^3/\mu L$)	0.16 ± 0.03[f]	0.18 ± 0.03[f]	0.15 ± 0.02[b]	0.13 ± 0.03[g]
Nucleated erythrocytes/100 leukocytes	0.40 ± 0.22	0.40 ± 0.16	0.80 ± 0.36	0.10 ± 0.10
Clinical Chemistry				
Urea nitrogen (mg/dL)	20.2 ± 0.8	20.5 ± 0.6	21.0 ± 0.9	21.6 ± 1.8[h]
Alanine aminotransferase (IU/L)	74 ± 3	67 ± 2	109 ± 7*	664 ± 60**
Sorbitol dehydrogenase (IU/L)	12 ± 1	9 ± 2	10 ± 1	60 ± 6*
Female				
n	10	9	10	10
Hematology				
Hematocrit (%)	43.9 ± 0.4	43.2 ± 0.3	40.5 ± 0.7**	38.0 ± 0.7**
Hemoglobin (g/dL)	16.9 ± 0.2	16.6 ± 0.2	15.7 ± 0.3**	14.7 ± 0.3**
Erythrocytes ($10^6/\mu L$)	7.84 ± 0.08	7.81 ± 0.07	7.70 ± 0.13	7.60 ± 0.14
Mean cell volume (fL)	55.9 ± 0.1	55.2 ± 0.2**	52.6 ± 0.2**	49.9 ± 0.3**
Mean cell hemoglobin (pg)	21.5 ± 0.1	21.3 ± 0.1	20.4 ± 0.1**	19.4 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	38.4 ± 0.1	38.5 ± 0.2	38.8 ± 0.2	38.8 ± 0.1
Leukocytes ($10^3/\mu L$)	4.91 ± 0.46	4.39 ± 0.34	3.76 ± 0.17	4.01 ± 0.21
Segmented neutrophils ($10^3/\mu L$)	1.04 ± 0.13	0.89 ± 0.14	0.69 ± 0.07*	0.66 ± 0.12*
Lymphocytes ($10^3/\mu L$)	3.77 ± 0.34	3.39 ± 0.24	2.99 ± 0.16	3.29 ± 0.17
Monocytes ($10^3/\mu L$)	0.14 ± 0.04[b]	0.10 ± 0.00[c]	0.10 [i]	0.10 ± 0.00[c]
Eosinophils ($10^3/\mu L$)	0.12 ± 0.02[b]	0.10 ± 0.03[d]	0.10 ± 0.00[b]	0.10 ± 0.00[f]
Nucleated erythrocytes/100 leukocytes	0.80 ± 0.29	0.50 ± 0.17[j]	0.10 ± 0.10*	0.20 ± 0.13
Clinical Chemistry				
Alanine aminotransferase (IU/L)	52 ± 2	45 ± 1	49 ± 3	94 ± 8**
Sorbitol dehydrogenase (IU/L)	7 ± 1	6 ± 0	6 ± 0	11 ± 1*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error
[b] n=6 [c] n=7 [d] n=4 [e] n=5 [f] n=8 [g] n=3 [h] n=2 [i] n=1; no standard error calculated [j] n=10

TABLE G2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male				
n	10	10	10	9
Hematology				
Hematocrit (%)	45.4 ± 1.4	46.0 ± 1.1	45.8 ± 0.5	44.2 ± 0.9
Hemoglobin (g/dL)	15.3 ± 0.3	15.1 ± 0.3	15.2 ± 0.2	14.8 ± 0.3
Erythrocytes ($10^6/\mu L$)	8.82 ± 0.24	8.89 ± 0.17	8.83 ± 0.12	8.74 ± 0.16
Mean cell volume (fL)	51.4 ± 0.5	51.7 ± 0.4	51.9 ± 0.5	50.5 ± 0.7
Mean cell hemoglobin (pg)	17.4 ± 0.1	17.0 ± 0.2*	17.2 ± 0.2	16.9 ± 0.4**
Mean cell hemoglobin concentration (g/dL)	33.9 ± 0.4	32.8 ± 0.5	33.2 ± 0.2	33.5 ± 0.6
Platelets ($10^3/\mu L$)	660.4 ± 20.6	676.6 ± 18.8	676.8 ± 12.2	690.9 ± 22.1
Reticulocytes ($10^6/\mu L$)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0*
Segmented neutrophils (%)	50.80 ± 2.37	50.70 ± 3.08	36.90 ± 1.88**	42.00 ± 3.74*
Lymphocytes (%)	46.10 ± 2.35	46.00 ± 2.88	59.70 ± 2.03	55.78 ± 4.03*
Monocytes (%)	1.10 ± 0.31	1.80 ± 0.36	1.10 ± 0.23	1.00 ± 0.29
Eosinophils (%)	2.00 ± 0.30	1.50 ± 0.27	2.20 ± 0.25	1.22 ± 0.36
Nucleated erythrocytes (/100 wbc)	1.10 ± 0.59	0.80 ± 0.39	2.50 ± 0.86	1.22 ± 0.47
Clinical Chemistry				
Urea nitrogen (mg/dL)	18.0 ± 0.4	19.1 ± 0.6	18.8 ± 0.4	18.8 ± 0.5
Creatinine (mg/dL)	0.60 ± 0.01	0.61 ± 0.01	0.62 ± 0.02	0.59 ± 0.01
Total protein (g/dL)	7.2 ± 0.1	7.3 ± 0.1	7.5 ± 0.1*	7.4 ± 0.1
Albumin (g/dL)	4.3 ± 0.1	4.2 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
Alkaline phosphatase (IU/L)	131 ± 4	118 ± 6[b]	121 ± 4	129 ± 4
Alanine aminotransferase (IU/L)	47 ± 2	46 ± 3	50 ± 4	108 ± 7**
Sorbitol dehydrogenase (IU/L)	7 ± 1	6 ± 2[b]	9 ± 1	16 ± 1**
Creatine kinase (IU/L)	94 ± 11	92 ± 13	87 ± 8	74 ± 7
Urinalysis				
Bile acids ($\mu mol/L$)	3.86 ± 1.12[c]	13.22 ± 6.47[b]	7.00 ± 1.98	4.56 ± 1.37

TABLE G2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study of C.I. Direct Blue 218 (continued)

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Female				
n	9	9	10	10
Hematology				
Hematocrit (%)	43.2 ± 0.8	43.2 ± 0.6	42.9 ± 0.7	41.6 ± 0.4*
Hemoglobin (g/dL)	15.2 ± 0.2	15.2 ± 0.1	14.9 ± 0.2	14.6 ± 0.2**
Erythrocytes ($10^6/\mu L$)	7.78 ± 0.16	7.86 ± 0.08	7.85 ± 0.10	7.80 ± 0.08
Mean cell volume (fL)	55.6 ± 0.9	55.0 ± 0.7	54.7 ± 0.5	53.3 ± 0.4*
Mean cell hemoglobin (pg)	19.6 ± 0.2	19.3 ± 0.1	19.0 ± 0.1*	18.7 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	35.2 ± 0.4	35.2 ± 0.3	34.7 ± 0.4	35.1 ± 0.3
Platelets ($10^3/\mu L$)	584.6 ± 11.9	582.9 ± 19.6	592.0 ± 19.8	551.7 ± 18.7
Reticulocytes ($10^6/\mu L$)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Segmented neutrophils (%)	26.00 ± 3.03	34.11 ± 4.02	34.00 ± 2.66	36.00 ± 2.70*
Lymphocytes (%)	70.56 ± 2.88	60.89 ± 3.81	62.40 ± 2.38	60.90 ± 2.53*
Monocytes (%)	1.78 ± 0.52	2.89 ± 0.42	2.30 ± 0.56	1.80 ± 0.55
Eosinophils (%)	1.56 ± 0.47	2.11 ± 0.42	1.30 ± 0.30	1.30 ± 0.37
Nucleated erythrocytes/100 leukocytes	1.67 ± 0.41	1.22 ± 0.28	0.60 ± 0.22	2.50 ± 0.58
Clinical Chemistry				
Urea nitrogen (mg/dL)	19.6 ± 0.4	19.5 ± 0.8	19.9 ± 0.7	22.1 ± 0.6*
Creatinine (mg/dL)	0.65 ± 0.02	0.63 ± 0.02	0.61 ± 0.02	0.62 ± 0.01
Total protein (g/dL)	8.6 ± 0.2	8.3 ± 0.1	8.4 ± 0.1	8.5 ± 0.2
Albumin (g/dL)	5.0 ± 0.1	5.1 ± 0.1	5.1 ± 0.2	5.2 ± 0.2
Alkaline phosphatase (IU/L)	138 ± 7	146 ± 5	129 ± 15	131 ± 7
Creatine kinase (IU/L)	88 ± 17	82 ± 20	89 ± 24	75 ± 13
Alanine aminotransferase (IU/L)	36 ± 1	41 ± 2	36 ± 3	73 ± 6**
Sorbitol dehydrogenase (IU/L)	7 ± 1	8 ± 1	8 ± 1	14 ± 1**
Urinalysis				
Bile acids (μmol/L)	47.33 ± 6.38	39.11 ± 7.08	43.00 ± 4.62	47.60 ± 10.87

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error
[b] n=9
[c] n=7

TABLE G3
Hematology and Clinical Chemistry Data for Mice in the 13-Week Feed Study of C.I. Direct Blue 218[a]

	0 ppm	3,000 ppm	10,000 ppm	20,000 ppm
Male				
n	10	9	9	10
Hematology				
Hematocrit (%)	38.3 ± 0.5	37.6 ± 0.3	36.4 ± 0.7*	33.7 ± 2.2**
Hemoglobin (g/dL)	15.6 ± 0.2	15.3 ± 0.1	14.8 ± 0.3*	14.0 ± 0.9*
Erythrocytes ($10^6/\mu L$)	7.81 ± 0.08	7.65 ± 0.06	7.62 ± 0.15	7.60 ± 0.45
Mean cell volume (fL)	49.2 ± 0.4	49.2 ± 0.3	47.8 ± 0.2**	44.0 ± 0.9**
Mean cell hemoglobin (pg)	20.0 ± 0.1	20.0 ± 0.1	19.4 ± 0.1**	18.3 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	40.7 ± 0.1	40.7 ± 0.2	40.7 ± 0.1	41.6 ± 0.4**
Leukocytes ($10^3/\mu L$)	4.13 ± 0.53	3.98 ± 0.59	5.23 ± 0.24	5.59 ± 0.51
Segmented neutrophils ($10^3/\mu L$)	0.74 ± 0.15	0.83 ± 0.20	0.83 ± 0.13	1.58 ± 0.34*
Lymphocytes ($10^3/\mu L$)	3.27 ± 0.42	3.08 ± 0.44	4.24 ± 0.18*	3.79 ± 0.48
Monocytes ($10^3/\mu L$)	0.12 ± 0.02[b]	0.10 ± 0.00[c]	0.13 ± 0.02[d]	0.16 ± 0.03[e]
Eosinophils ($10^3/\mu L$)	0.10 ± 0.00[b]	0.10 ± 0.00[f]	0.17 ± 0.07[g]	0.16 ± 0.04[c]
Clinical Chemistry				
Urea nitrogen (mg/dL)	23.9 ± 1.9	23.8 ± 1.8	27.2 ± 1.0	26.7 ± 2.1
Alanine aminotransferase (IU/L)	82 ± 11	92 ± 14	119 ± 9*	223 ± 25**
Sorbitol dehydrogenase (IU/L)	35 ± 2	38 ± 2	83 ± 9**	175 ± 12**[h]
Female				
n	10	10	10	10
Hematology				
Hematocrit (%)	38.3 ± 0.4	38.2 ± 0.6	38.0 ± 0.4	36.4 ± 1.2
Hemoglobin (g/dL)	15.4 ± 0.2	15.4 ± 0.2	15.3 ± 0.2	14.8 ± 0.5
Erythrocytes ($10^6/\mu L$)	7.52 ± 0.09	7.54 ± 0.09	7.85 ± 0.09*	7.66 ± 0.24*
Mean cell volume (fL)	51.0 ± 0.3	50.6 ± 0.4	48.4 ± 0.3**	47.6 ± 0.3**
Mean cell hemoglobin (pg)	20.5 ± 0.2	20.4 ± 0.1	19.5 ± 0.1**	19.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	40.1 ± 0.1	40.3 ± 0.2	40.2 ± 0.2	40.6 ± 0.2
Leukocytes ($10^3/\mu L$)	4.13 ± 0.46	5.97 ± 0.68	5.94 ± 0.62	5.69 ± 0.69
Segmented neutrophils ($10^3/\mu L$)	0.65 ± 0.10	0.89 ± 0.16	1.32 ± 0.22*	0.96 ± 0.21
Lymphocytes ($10^3/\mu L$)	3.34 ± 0.37	4.83 ± 0.54	4.42 ± 0.41	4.54 ± 0.60
Monocytes ($10^3/\mu L$)	0.12 ± 0.02[b]	0.17 ± 0.03	0.20 ± 0.03[d]	0.20 ± 0.06[e]
Eosinophils ($10^3/\mu L$)	0.13 ± 0.03[i]	0.23 ± 0.09[g]	0.14 ± 0.04[c]	0.13 ± 0.03[g]
Clinical Chemistry				
Urea nitrogen (mg/dL)	21.1 ± 1.4	19.8 ± 1.3	18.4 ± 1.2	23.4 ± 1.6
Alanine aminotransferase (IU/L)	71 ± 9	73 ± 5	138 ± 13**	202 ± 17**[h]
Sorbitol dehydrogenase (IU/L)	27 ± 1	31 ± 1*	83 ± 5**	165 ± 18**

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error
[b] n=6 [c] n=5 [d] n=7 [e] n=8 [f] n=2 [g] n=3 [h] n=9 [i] n=4

TABLE G4
Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluation in the 2-Year Feed Study of C.I. Direct Blue 218[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male				
n	9	10	10	10
Hematology				
Hematocrit (%)	48.9 ± 1.4	45.2 ± 0.8*	46.7 ± 1.4	43.7 ± 0.8**
Hemoglobin (g/dL)	16.9 ± 0.3	16.0 ± 0.3*	16.1 ± 0.3*	15.8 ± 0.2**
Erythrocytes (10^6/μL)	10.48 ± 0.27	9.60 ± 0.24	10.12 ± 0.36	9.73 ± 0.24
Mean cell volume (fl)	46.6 ± 0.4	47.0 ± 0.7	46.4 ± 0.7	45.0 ± 0.6**
Mean cell hemoglobin concentration (g/dL)	34.7 ± 0.8	35.5 ± 0.3	34.6 ± 0.6	36.2 ± 0.3
Platelets (10^3/μL)	1,543.0 ± 104.0	1,467.0 ± 79.0	1,640.0 ± 91.0	1,680.0 ± 122.0
Reticulocytes (10^6/μL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Leukocytes (10^3/μL)	4.72 ± 0.71	4.55 ± 0.62	5.37 ± 0.40	5.44 ± 0.83
Segmented neutrophils (10^3/μL)	1.60 ± 0.40	1.90 ± 0.29	1.98 ± 0.28	2.27 ± 0.45
Lymphocytes (10^3/μL)	3.01 ± 0.38	2.51 ± 0.36	3.30 ± 0.25	3.12 ± 0.52
Monocytes (10^3/μL)	0.10 ± 0.04	0.07 ± 0.02	0.08 ± 0.03	0.05 ± 0.02
Eosinophils (10^3/μL)	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
Clinical Chemistry				
Alanine aminotransferase (IU/L)	46 ± 9[b]	34 ± 4[c]	27 ± 2[d]	60 ± 5**[c]
Sorbitol dehydrogenase (IU/L)	46 ± 7	39 ± 2	38 ± 3	54 ± 1**
Female				
n	10	10	10	10
Hematology				
Hematocrit (%)	45.4 ± 0.6	44.8 ± 1.0	46.2 ± 0.8	43.1 ± 0.8*
Hemoglobin (g/dL)	16.2 ± 0.1	16.1 ± 0.3	16.4 ± 0.2	15.5 ± 0.2*
Erythrocytes (10^6/μL)	9.91 ± 0.08	9.77 ± 0.18	10.29 ± 0.22	9.80 ± 0.18
Mean cell volume (fL)	45.7 ± 0.5	46.1 ± 0.2	44.9 ± 0.2*	44.0 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	35.7 ± 0.4	36.0 ± 0.3	35.5 ± 0.4	35.9 ± 0.4
Platelets (10^3/μL)	1,273.0 ± 38.0	1,340.0 ± 93.0	1,186.0 ± 34.0	1,363.0 ± 55.0
Reticulocytes (10^6/μL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Leukocytes (10^3/μL)	4.41 ± 0.32	4.79 ± 0.49	5.68 ± 0.57	6.45 ± 0.65*
Segmented neutrophils (10^3/μL)	1.50 ± 0.17	1.75 ± 0.21	2.05 ± 0.39	2.10 ± 0.29
Lymphocytes (10^3/μL)	2.86 ± 0.21	2.95 ± 0.35	3.50 ± 0.31	4.27 ± 0.52*
Monocytes (10^3/μL)	0.02 ± 0.01	0.05 ± 0.02	0.11 ± 0.04**	0.07 ± 0.02*
Eosinophils (10^3/μL)	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Clinical Chemistry				
Alanine aminotransferase (IU/L)	30 ± 4	32 ± 3	28 ± 4	64 ± 4**[d]
Sorbitol dehydrogenase (IU/L)	28 ± 3	27 ± 2	29 ± 1	61 ± 5**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error
[b] n=7
[c] n=9
[d] n=8

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATIONS STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of C.I. Direct Blue 218

C.I. Direct Blue 218 was obtained in two lots. The first lot (AT101681) was obtained from the Atlantic Chemical Company (Nutley, NJ). Based on the analysis of lot AT101681 originally supplied, desalting was necessary to reduce the inorganic salt content to a level acceptable for bioassay. Lot AT101681 was desalted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), using a dialysis procedure. The salt content (expressed as sodium chloride) was reduced from approximately 16.5% to about 1.7%, a salt reduction of 90% based on elemental analysis. The purified chemical was then milled to a fine powder and manually homogenized, and the desalted material was assigned lot number M042382. The second lot (F47238) was obtained from Ciba-Geigy Corporation Dyestuffs and Chemical Division (Toms River, NJ) then desalted by the Atlantic Chemical Company using a dialysis procedure; the resultant salt content was 0.7%. Lot M042382 was used throughout the 14-day and 13-week studies in rats and mice and lot F47238 was used throughout the 2-year studies in rats and mice. Reports on identity, purity, and stability analyses performed by the analytical chemistry laboratory in support of the C.I. Direct Blue 218 studies are on file at the National Institute of Environmental Health Sciences.

Lots M042382 and F47238 of the dye, a dark blue powder, were identified as C.I. Direct Blue 218 by infrared and ultraviolet/visible spectroscopy. Nuclear magnetic resonance (NMR) spectroscopic analysis was unsuccessful due to the apparent paramagnetic interferences from copper in the sample. All spectra were consistent with those expected for the structure (Figures H1, H2, and H3) and similar to those of C.I. Direct Blue 15 from which C.I. Direct Blue 218 is synthesized.

The purity of lots M042382 and F47238 was determined by elemental analyses, weight loss on drying, reduction titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Reduction titration was performed in acetic acid containing titanium (III) chloride with standardized ferric ammonium sulfate. Thin-layer chromatography (TLC) was performed on silica gel plates using two solvent systems: 1) methylethyl ketone:toluene:diethylamine:pyridine:water (26:11:21:21:21), and 2) diethylamine:water (85:15). Visualization was accomplished with visible light and short (254 nm) and long (366 nm) wavelength ultraviolet light. HPLC was performed with a Whatman CO:PELL ODS column in a mixture of two solvents: A) 0.01M tetrabutylammonium hydroxide (TBA) in water, adjusted to a pH of 6.8 with 10% phosphoric acid, and B) 0.01 M TBA in methanol, adjusted to the same pH as solvent A using the same method as for solvent A, at a flow rate of 1 mL/minute. Visible detection was at 658 nm and ultraviolet detection was at 254 nm.

Elemental analyses could not be used to confirm the identity or relative purity of the major component in either lot because each sample was a complex mixture of organic and inorganic components. However, elemental analyses indicated the presence of 1.7% sodium chloride in lot M042382 and 0.68% sodium chloride in lot F47238. Elemental analysis of copper (9%) was consistent with the presence of two copper atoms for each organic molecule. Inorganic copper salt could have been present but this could not be confirmed. Weight loss on drying for lot M042382 indicated 6.07% ± 0.00% water and 11.38% ± 0.02% water in lot F47238. Reduction titration indicated a purity of 90.8% ± 1.1% for lot M042382 and a purity of 83% ± 2% for lot F47238, assuming reduction of two azo groups and two copper ions per molecule of dye. These values are probably enhanced by the presence of titrable impurities. Thin-layer chromatography using system 1 indicated one major spot, ten minor impurities, and two trace impurities and one slight trace impurity for lot M042382 and one major spot, six minor impurities, and two trace impurities for lot F47238. Solvent system 2 indicated one major spot, five minor impurities, and two trace impurities for lot M042328 and one major spot, three minor impurities, and three trace impurities for lot F47238. HPLC indicated a major peak and up to nine impurities (peaks > 1% of total area) with

combined peak areas of approximately 34% at 254 nm and 25% at 658 nm relative to that of the major peak (Figure H2). The combined data for lot M042382 and F47238 provides a final estimate of approximately 60% by weight for the major component for each lot.

As a supplement to the identity and purity analyses, solvent extractions were performed to determine the concentrations of 3,3′-dimethoxybenzidine and benzidine in lots M042382 and F47238. HPLC indicated 7.4 ppm 3,3′-dimethoxybenzidine in lot M042382 and less than 1 ppm in lot F47238. Benzidine was not present at levels greater than 1 ppm in either lot.

Stability studies performed by HPLC using the system described above, but with acetophenone added as an internal standard and ultraviolet detection at 254 nm indicated that C.I. Direct Blue 218, when stored protected from light, was stable as a bulk chemical for 2 weeks at temperatures up to 25° C. The stability of the bulk chemical was also monitored periodically at the study laboratory by ultraviolet/visible spectrophotometry and HPLC. No degradation of the study material was seen throughout the studies.

Preparation and Analysis of Dose Formulations

Initial attempts were made to use water as the vehicle for dose formulations. However, C.I. Direct Blue 218 solubility in water was limited. Attempts to define the solubility were unsuccessful. The absolute solubility could not be determined because of the complex nature of this commerical dye, i.e. the dye is a mixture of different complex species with different solubilities. Subsequently, the dose formulations were prepared by mixing C.I. Direct Blue 218 with feed (Table H1). Dose formulations were prepared prior to the initiation and at the midpoint of the 14-day studies, prior to the initiation and every 2 weeks for the 13-week studies, and every 2 weeks in the 2-year studies.

Dose formulation homogeneity and stability analyses of the 500 ppm concentration were conducted by the analytical chemistry laboratory. C.I. Direct Blue 218 exhibited a broad absorption spectrum with a maximum at 622 nm. The formulations were extracted with 90 mL of methanol, centrifuged, and diluted with methanol. The samples were filtered and the absorbance was measured at 622 nm. Homogeneity was confirmed and the stability of the dose formulations was established for 3 weeks when stored in the dark at temperatures up to 25° C and for 1 week when stored open to air and light.

Periodic analyses of the dose formulations of C.I. Direct Blue 218 were conducted at the study laboratory and at the analytical chemistry laboratory using visible spectroscopy. The feed was extracted with methanol containing 1 M tetrabutylammonium hydroxide. The absorbance was determined at 622 nm. During the 14-day studies, the dose formulations were analyzed prior to study initiation (Table H2). During the 13-week studies, the dose formulations were analyzed at the initiation, midpoint, and termination of the studies (Table H3). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks (Table H4). In the 2-year studies, 92 of 93 dose formulations analyzed were within 10% of the target concentration. Results of periodic referee analysis performed by the analytical chemistry laboratory were in good agreement with the results obtained by the study laboratory (Table H5).

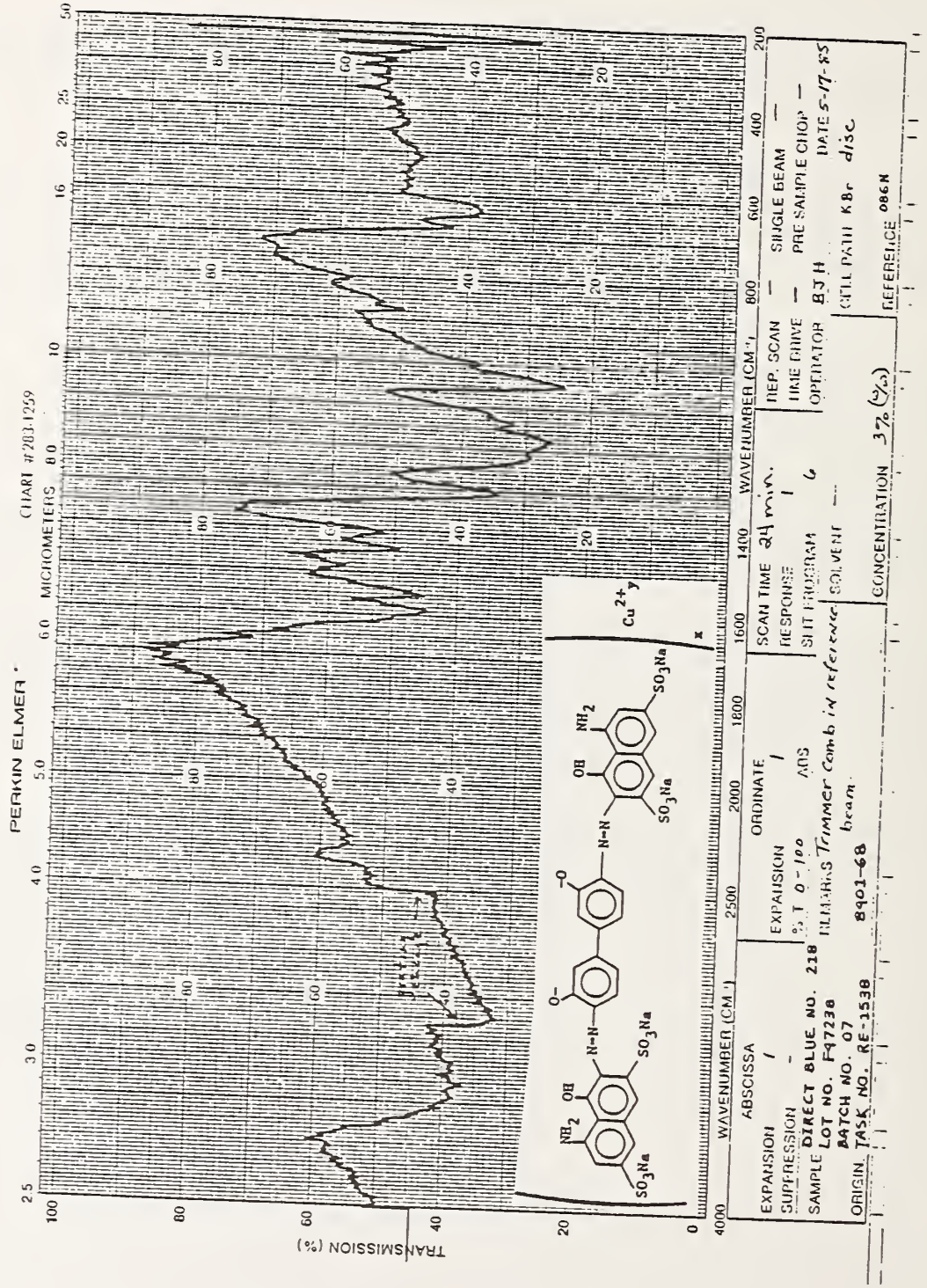

FIGURE H1
Infrared Absorption Spectrum of C.I. Direct Blue 218

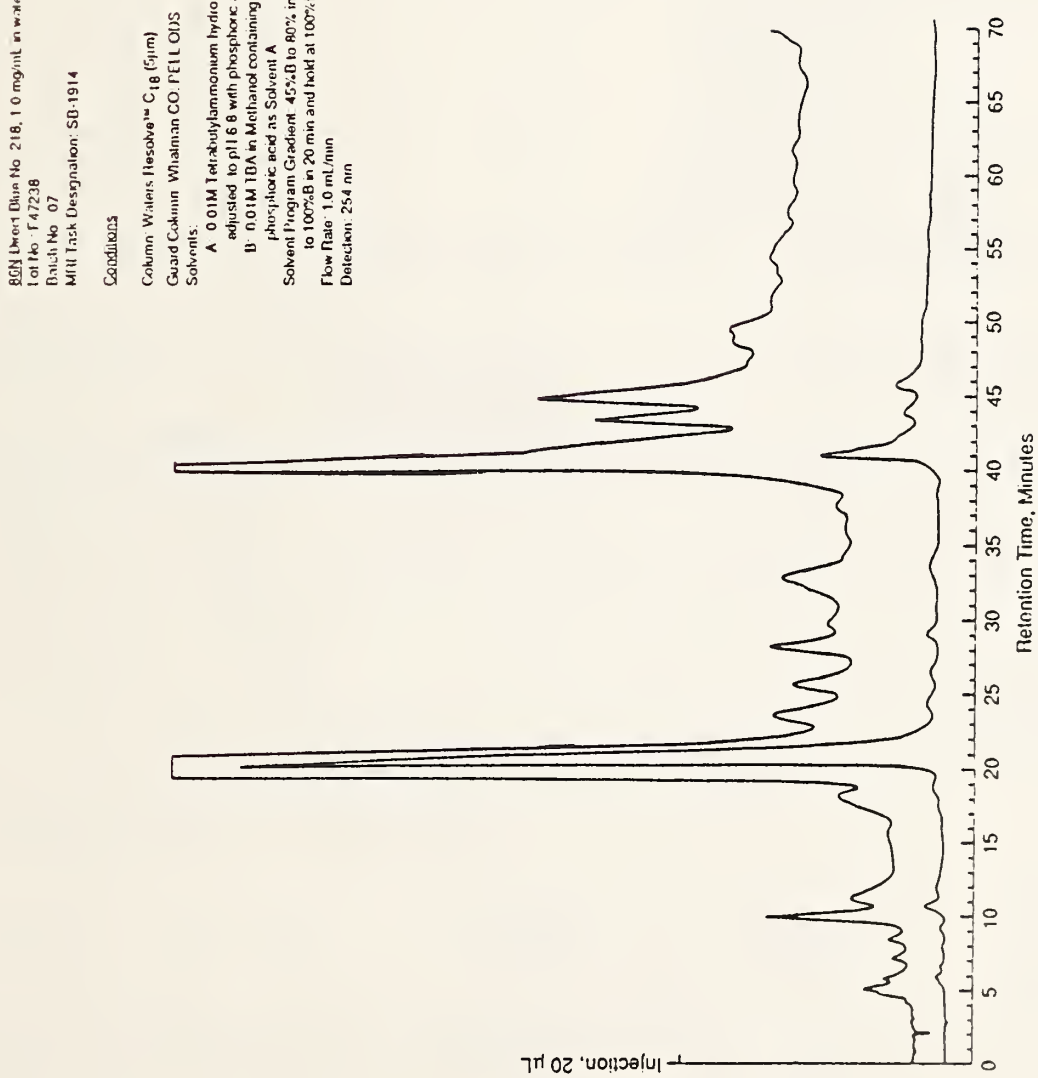

FIGURE H2
High Performance Liquid Chromatograph of C.I. Direct Blue 218 at 254 nm

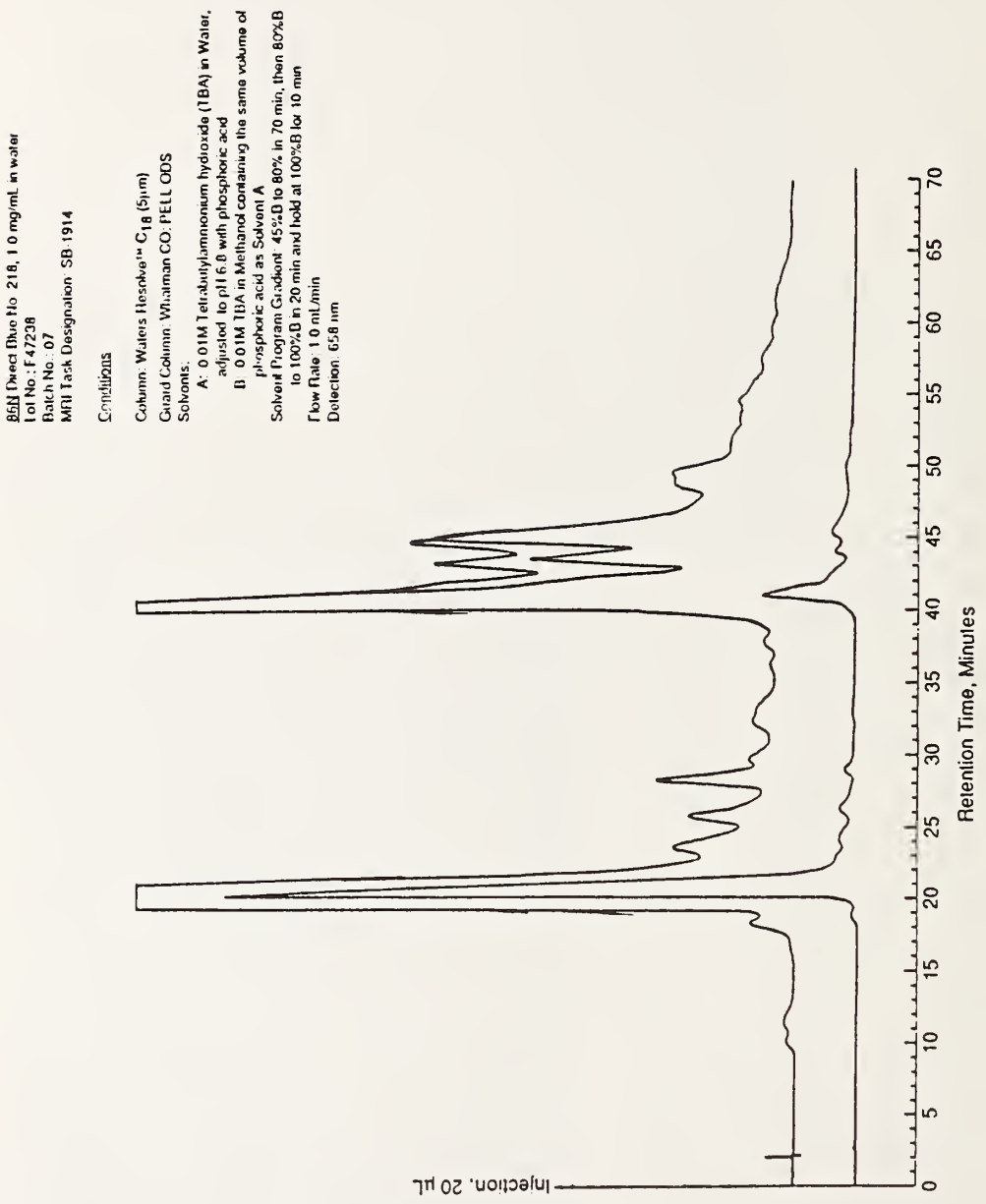

FIGURE H3
High Performance Liquid Chromatograph of C.I. Direct Blue 218 at 658 nm

TABLE H1
Preparation and Storage of Dose Formulations in the Feed Studies of C.I. Direct Blue 218

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
Premix was prepared by mixing C.I. Direct Blue 218 and feed with mortar and pestle; premix and remaining feed layered in a twin shell blender and mixed for 10 minutes.	Premix was prepared by mixing C.I. Direct Blue 218 and feed; premix and remaining feed layered in a twin shell blender and mixed for 10 minutes with intensifier bar on.	Premix was prepared by mixing C.I. Direct Blue 218 and feed; premix and remaining feed layered in a twin shell blender and mixed for 5 minutes with intensifier bar on and 10 minutes with intensifier bar off.
Lot Number		
M042382	Same as 14-day studies	F47238
Maximum Storage Time		
2 weeks	Same as 14-day studies	3 weeks after date of preparation
Storage		
In the dark at 5° C	In the dark under refrigeration at a temperature of 1° C	In double-sealed plastic bags, at or below room temperature
Study Laboratory		
International Research and Development Corporation, Mattawam, MI	Same as 14-day studies	Microbiological Associates, Incorporated, Bethesda, MD
Analytical Chemistry Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 14-day studies	Same as 14-day studies

TABLE H2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 14-Day Feed Studies of C.I. Direct Blue 218

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
30 June 1982	1 July 1982	1,000	1,030	+3
		3,000	2,980	−1
		15,000	14,600	−3
		30,000	29,500	−2
	8 July 1982	7,000	7,590	+8

[a] Results of duplicate analyses

TABLE H3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of C.I. Direct Blue 218

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
7 December 1982	9 December 1982	20,000	22,600[b]	+13
		20,000	22,100[c]	+11
		20,000	21,700[d]	+9
	10 December 1982	3,000	2,990[b]	0
		3,000	3,040[c]	+1
		3,000	2,970[d]	–1
13 December 1982	14 December 1982	20,000	19,200[b]	–4
		20,000	19,700[c]	–1
		20,000	19,800[d]	–1
	15 December 1982	3,000	3,030[b]	+1
		3,000	3,080[c]	+3
		3,000	2,910[d]	–3
15 December 1982	15 December 1982	10,000	9,430	–6
10 January 1983	14 January 1983	3,000	2,910	–3
24 January 1983	25 January 1983	3,000	2,990	0
	26 January 1983	10,000	9,480	–5
		20,000	19,100	–4
7 March 1983	8 March 1983	10,000	10,400	+4

[a] Results of duplicate analyses
[b] Sample taken from top of blender
[c] Sample taken from middle of blender
[d] Sample taken from bottom of blender

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of C.I. Direct Blue 218

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
9 August 1985	12 August 1985	1,000	973[b]	–3
		1,000	1,016[c]	+2
		1,000	1,004[d]	0
		3,000	2,920	–3
		10,000	10,600[b]	+6
		10,000	9,980[c]	0
		10,000	9,870[d]	–1
2 October 1985	3 October 1985	10,000	9,950	–1
		10,000	9,840	–2
		10,000	10,000	0
10 December 1985	12 December 1985	1,000	1,090	+9
		1,000	1,070	+7
		1,000	1,060	+6
		3,000	3,030	+1
		3,000	3,030	+1
		3,000	3,030	+1
		10,000	9,850	–2
		10,000	9,840	–2
		10,000	9,860	–1
28 January 1986	29 January 1986	1,000	1,030	+3
		3,000	2,860	–5
		10,000	9,950	–1
20 March 1986	24 March 1986	1,000	1,040	+4
		3,000	2,890	–4
		10,000	9,410	–6
8 May 1986	9 May 1986	1,000	933	–7
		1,000	1,090	+9
		3,000	2,980	–1
		3,000	3,280	+9
		10,000	9,830	–2
		10,000	10,100	+1
17 July 1986	18-20 July 1986	1,000	1,080	+8
		1,000	1,090	+9
		3,000	3,180	+6
		3,000	3,280	+9
		10,000	10,600	+6
		10,000	10,800	+8
17 September 1986	19 September 1986	1,000	951	–5
		1,000	943	–6
		3,000	2,760	–8
		3,000	2,720	–9
		10,000	9,570	–4
		10,000	9,140	–9

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of C.I. Direct Blue 218 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
17 November 1986	21 November 1986	1,000	1,000	0
		1,000	986	–1
		3,000	3,030	+1
		3,000	3,120	+4
		10,000	9,910	–1
		10,000	9,750	–3
6 January 1987	7-8 January 1987	1,000	1,090[e]	+9
		1,000	1,070	+7
		3,000	3,110	+4
		3,000	3,150	+5
		10,000	10,600	+6
		10,000	10,600	+6
2 March 1987	3-4 March 1987	1,000	1,000	0
		1,000	1,070	+7
		3,000	3,160	+5
		3,000	3,150	+5
		10,000	10,800	+8
		10,000	10,400	+4
21 April 1987	22 April 1987	1,000	919	–8
		1,000	964	–4
		3,000	3,000	0
		3,000	2,950	–2
		10,000	9,950	–1
		10,000	10,100	+1
22 June 1987	23 June 1987	1,000	1,130	+13
		1,000	1,100	+10
		3,000	3,260	+9
		3,000	3,230	+8
		10,000	10,500	+5
		10,000	10,400	+4
24 June 1987	24 June 1987	1,000	998[f]	0
18 August 1987	18 August 1987	1,000	963[f]	–4
4 February 1988[g]	4-5 February 1988	1,000	1,010	+1
		1,000	1,000	0
		3,000	2,870	–4
		3,000	3,080	+3
		10,000	10,300	+3
		10,000	10,800	+8

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of C.I. Direct Blue 218 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
24 March 1988[g]	25 March 1988	1,000	1,030	+3
		1,000	1,000	0
		3,000	3,030	+1
		3,000	3,030	+1
		10,000	10,400	+4
		10,000	10,200	+2
20 April 1988[g]	26-27 April 1988	1,000	1,050	+5
		1,000	993	−1
		3,000	3,220	+7
		3,000	3,040	+1
		10,000	10,700	+7
		10,000	10,600	+6

[a] Results of duplicate analyses
[b] Sample taken from top right of blender
[c] Sample taken from top left of blender
[d] Sample taken from bottom of blender
[e] Results of triplicate analyses
[f] Results of remix
[g] Doses administered to rats only

TABLE H5
Results of Referee Analysis of Dose Formulations in the 13-Week and 2-Year Feed Studies of C.I. Direct Blue 218

Date Prepared	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
13-Week Studies			
10 January 1983	3,000	2,910	4,107 ± 110
7 March 1983	10,000	10,400	10,667 ± 569
2-Year Studies			
9 August 1985	1,000	998	975 ± 59
20 March 1986	3,000	2,890	3,097 ± 69
17 September 1986	10,000	9,570	9,853 ± 130
2 March 1987	3,000	3,150	2,933 ± 49
18 August 1987	1,000	963	1,049 ± 21
4 February 1988	10,000	10,300	10,100 ± 153

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard deviation)

APPENDIX I
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES

TABLE I1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of C.I. Direct Blue 218

Week	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	14.2	156	13.7	154	89	14.1	156	272	14.1	154	917
5	15.1	237	14.6	231	63	15.7	233	201	15.6	234	669
10	14.7	304	15.8	312	50	16.3	318	154	16.3	311	524
13	16.0	343	15.5	340	46	15.4	346	133	15.0	336	447
17	15.9	379	16.2	364	44	15.4	378	122	15.3	360	425
21	16.3	399	16.2	388	42	16.1	397	122	16.0	371	432
25	18.3	411	17.5	405	43	18.9	407	139	18.5	378	489
29	20.7	425	20.4	422	48	19.6	433	136	18.0	401	447
33	20.3	436	18.4	432	43	18.6	444	126	17.1	409	417
37	18.4	446	16.9	440	38	18.3	448	123	18.0	410	439
41	17.7	452	18.5	444	42	17.5	453	116	17.8	408	436
45	16.8	456	16.8	449	37	17.2	450	115	16.2	415	390
49	16.7	466	16.5	454	36	16.6	462	108	16.9	419	402
53	17.0	465	16.5	454	36	17.0	462	111	16.5	420	393
57	18.4	467	17.6	454	39	18.2	458	119	17.1	415	414
61	18.2	461	17.9	447	40	17.8	456	117	18.4	414	443
65	17.7	466	16.1	454	35	16.5	456	109	16.0	413	388
69	18.2	469	18.3	456	40	17.3	458	113	16.4	420	392
73	17.3	466	17.5	459	38	17.3	455	114	16.2	410	394
77	17.0	465	17.2	458	38	17.3	461	112	16.3	420	387
80	16.3	463	16.0	458	35	15.7	457	103	15.5	416	374
85	15.7	460	15.0	454	33	15.5	457	102	13.7	411	334
89	17.1	455	14.6	441	33	15.5	442	105	15.6	405	386
93	17.6	454	15.2	434	35	13.8	449	92	14.9	403	370
97	16.4	450	15.4	451	34	15.5	445	105	15.4	401	384
101	16.6	453	15.1	439	34	16.3	443	111	14.3	387	369
Mean for weeks											
1-13	15.0	260	14.9	259	62	15.4	263	190	15.3	259	639
14-52	17.9	430	17.5	422	42	17.6	430	123	17.1	397	431
53-101	17.2	461	16.3	451	36	16.4	454	109	15.9	410	387

[a] Grams of feed consumed per animal per day
[b] Milligrams of C.I. Direct Blue 218 consumed per day per kilogram body weight

TABLE I2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of C.I. Direct Blue 218

Week	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	10.5	119	10.2	118	86	10.3	119	260	10.4	120	866
5	10.5	148	10.1	150	67	9.8	149	197	9.2	152	605
10	9.9	181	9.8	184	53	9.9	185	160	9.3	180	519
13	9.4	191	9.2	191	48	9.0	192	140	8.7	185	472
17	8.4	204	8.7	202	43	10.0	201	149	9.2	192	481
21	10.0	209	10.0	210	48	9.7	208	140	9.6	193	497
25	11.0	209	10.7	214	50	10.9	209	157	10.9	193	565
29	11.2	223	11.1	225	49	10.9	222	147	10.5	208	508
33	11.2	231	10.7	233	46	11.5	230	150	11.5	214	536
37	11.2	237	10.6	237	45	10.9	233	140	11.0	217	508
41	10.5	242	10.7	242	44	11.2	240	140	10.9	226	482
45	10.9	251	10.6	247	43	10.9	244	134	11.3	232	487
49	11.3	258	11.1	259	43	10.9	254	129	10.8	244	445
53	11.5	269	11.6	267	44	11.7	261	135	11.9	249	479
57	11.1	275	11.9	275	43	11.4	270	127	11.7	259	450
61	11.5	283	12.2	273	45	11.4	275	124	11.6	263	442
65	12.1	292	12.0	290	41	12.4	289	129	12.5	276	453
69	12.9	304	13.7	306	45	12.6	305	124	12.1	288	421
73	12.6	314	12.5	311	40	12.5	310	121	12.0	294	409
77	12.4	322	12.8	318	40	12.8	317	121	12.8	302	424
80	13.0	332	12.9	329	39	12.8	327	118	12.1	310	389
85	12.4	336	12.4	334	37	12.0	334	107	11.9	312	382
89	11.9	334	11.4	331	34	11.6	333	105	10.4	308	337
93	11.9	337	12.4	336	37	12.0	332	109	11.9	309	385
97	11.3	341	12.3	342	36	12.8	337	114	11.9	316	376
101	12.5	346	11.7	340	35	12.3	338	109	11.1	310	358
Mean for weeks											
1-13	10.0	160	9.8	161	64	9.8	162	189	9.4	159	616
14-52	10.6	229	10.5	230	46	10.8	227	143	10.6	213	501
53-101	12.1	314	12.3	312	40	12.2	310	119	11.8	298	408

[a] Grams of feed consumed per animal per day
[b] Milligrams of C.I. Direct Blue 218 consumed per day per kilogram body weight

TABLE I3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Week	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	5.6	22.1	4.1	21.6	188	3.5	21.2	497	5.1	20.8	2,459
6	5.2	26.1	4.4	25.4	172	4.4	24.9	531	5.3	23.6	2,250
10	5.3	28.2	5.5	27.9	198	5.6	27.3	619	6.7	25.4	2,641
13	4.7	31.0	4.3	30.3	140	4.4	29.3	455	4.9	26.8	1,829
18	4.9	33.8	4.9	33.1	149	5.1	32.5	469	5.8	29.0	1,991
21	5.1	35.7	4.9	34.9	141	5.2	34.5	451	6.0	31.1	1,939
25	4.0	38.0	3.7	37.0	100	3.7	36.4	307	4.0	32.0	1,238
29	4.5	38.5	4.6	37.3	124	4.2	36.9	343	4.6	32.9	1,411
33	4.4	40.4	4.3	39.3	111	4.4	38.7	344	4.8	33.8	1,415
37	4.1	41.2	4.4	40.9	108	4.2	39.5	318	4.2	35.5	1,178
41	4.2	43.2	4.2	42.0	100	3.9	41.1	286	4.3	36.1	1,181
45	4.2	43.9	4.2	43.2	97	4.0	42.1	283	4.0	37.1	1,076
49	4.1	45.7	3.9	43.8	90	3.9	43.3	272	4.1	37.8	1,096
53	4.2	46.0	4.1	44.2	92	3.9	43.7	268	4.0	38.5	1,035
57	4.5	45.2	4.4	44.0	101	4.5	43.5	311	4.6	39.5	1,176
61	4.3	45.7	4.3	44.7	97	4.1	44.6	277	4.3	39.1	1,112
69	4.9	46.7	4.9	45.7	107	4.9	44.4	332	4.7	38.6	1,221
73	5.0	47.0	4.7	46.3	102	4.8	44.2	328	5.0	38.2	1,320
77	5.1	46.9	4.9	46.7	105	5.1	45.2	338	5.9	38.6	1,520
81	5.3	47.2	5.0	46.4	108	5.1	45.3	338	5.8	38.3	1,522
85	5.2	47.8	4.8	46.1	104	4.8	44.8	319	5.7	38.2	1,497
89	5.5	44.8	4.9	45.0	110	5.1	43.9	350	6.0	36.9	1,630
93	4.9	44.9	4.7	44.0	107	4.7	43.3	324	5.4	36.3	1,480
97	4.6	43.5	4.8	43.2	111	4.7	42.1	335	5.7	35.1	1,638
101	5.1	41.3	4.5	40.6	111	4.7	40.3	347	5.9	33.5	1,749
Mean for weeks											
1-13	5.2	26.9	4.5	26.3	174	4.5	25.7	526	5.5	24.2	2,295
14-52	4.4	40.0	4.4	39.1	113	4.3	38.3	341	4.6	33.9	1,392
53-101	4.9	45.6	4.7	44.7	105	4.7	43.8	322	5.3	37.6	1,408

[a] Grams of feed consumed per animal per day
[b] Milligrams of C.I. Direct Blue 218 consumed per day per kilogram body weight

TABLE I4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of C.I. Direct Blue 218

Week	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	5.8	18.5	4.1	18.2	223	5.8	18.2	960	5.7	17.5	3,256
6	5.7	22.4	4.8	21.7	223	5.9	20.8	858	6.1	19.9	3,083
10	6.1	25.1	6.5	24.9	259	6.1	24.0	763	6.2	22.1	2,792
13	5.8	27.2	4.5	26.4	171	5.2	25.6	604	5.6	23.5	2,374
18	5.6	30.3	6.1	29.1	210	7.2	27.7	777	7.4	25.5	2,919
21	6.3	32.8	6.3	31.2	201	6.8	29.7	688	6.9	27.5	2,499
25	4.1	35.2	4.3	34.7	124	4.9	31.8	463	5.1	29.3	1,748
29	5.1	35.9	5.8	36.1	160	4.9	33.4	438	6.4	30.4	2,092
33	4.9	37.6	5.0	38.3	130	5.5	35.4	464	6.0	31.6	1,907
37	4.4	39.4	4.8	39.9	120	4.8	35.9	399	5.1	32.8	1,551
41	4.5	40.3	4.5	40.6	112	4.4	38.0	344	4.9	33.0	1,482
45	4.5	40.9	4.6	42.0	108	4.6	39.0	353	4.7	33.8	1,382
49	4.5	43.3	4.6	42.9	107	4.6	41.1	337	4.7	34.8	1,337
53	4.5	43.8	4.8	44.1	108	4.5	42.4	319	4.8	35.4	1,347
57	5.2	44.1	5.3	45.0	119	5.2	42.5	365	5.7	36.1	1,568
61	4.9	44.5	4.9	45.2	107	4.9	42.8	347	5.7	35.4	1,606
69	5.5	46.5	5.8	45.6	127	5.9	44.2	400	6.4	35.4	1,813
73	5.2	47.2	5.6	45.4	124	5.7	44.1	385	6.1	35.3	1,721
77	5.6	47.2	6.0	46.4	128	6.0	44.8	401	6.9	35.4	1,960
81	6.2	46.1	6.2	45.9	136	6.1	44.4	412			
85	5.6	46.4	6.2	46.1	135	5.8	44.2	395	7.4	34.4	2,148
89	5.8	44.5	6.0	44.9	133	5.8	43.2	400	7.6	33.6	2,258
93	6.0	44.8	6.0	43.3	139	5.7	42.9	399	7.2	32.6	2,202
97	5.1	43.6	5.5	42.6	129	5.7	40.6	419	7.3	30.9	2,362
101	5.3	40.3	6.0	40.3	148	5.6	38.2	437	7.8	28.6	2,712
Mean for weeks											
1-13	5.9	23.3	5.0	22.8	219	5.8	22.2	796	5.9	20.8	2,876
14-52	4.9	37.3	5.1	37.2	141	5.3	34.7	474	5.7	31.0	1,879
53-101	5.4	44.9	5.7	44.6	128	5.6	42.9	390	6.6	33.9	1,973

[a] Grams of feed consumed per animal per day
[b] Milligrams of C.I. Direct Blue 218 consumed per day per kilogram body weight

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE J1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE J2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.54 ± 0.71	21.30 – 24.00	30
Crude Fat (% by weight)	5.47 ± 0.29	4.80 – 6.00	30
Crude Fiber (% by weight)	3.38 ± 0.29	2.80 – 3.90	30
Ash (% by weight)	6.61 ± 0.88	2.41 – 7.89	30
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.060	1.210 – 1.390	8
Cystine	0.306 ± 0.084	0.181 – 0.400	8
Glycine	1.150 ± 0.047	1.060 – 1.210	8
Histidine	0.576 ± 0.024	0.531 – 0.607	8
Isoleucine	0.917 ± 0.029	0.881 – 0.944	8
Leucine	1.946 ± 0.055	1.850 – 2.040	8
Lysine	1.270 ± 0.058	1.200 – 1.370	8
Methionine	0.448 ± 0.128	0.306 – 0.699	8
Phenylalanine	0.987 ± 0.140	0.665 – 1.110	8
Threonine	0.877 ± 0.042	0.824 – 0.940	8
Tryptophan	0.236 ± 0.176	0.107 – 0.671	8
Tyrosine	0.676 ± 0.105	0.564 – 0.794	8
Valine	1.103 ± 0.040	1.050 – 1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830 – 2.570	7
Linolenic	0.280 ± 0.040	0.210 – 0.320	7
Vitamins			
Vitamin A (IU/kg)	7,434 ± 3,558	4,500 – 19,000	30
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	37.95 ± 9.406	22.50 – 48.90	8
Thiamine (ppm)	20.30 ± 2.31	15.0 – 25.0	30
Riboflavin (ppm)	7.92 ± 0.87	6.10 – 9.00	8
Niacin (ppm)	103.38 ± 26.59	65.0 – 150.0	8
Pantothenic Acid (ppm)	29.54 ± 3.60	23.0 – 34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60 – 14.0	8
Folic Acid (ppm)	2.25 ± 0.73	1.80 – 3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19 – 0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6 – 65.0	8
Choline (ppm)	3,089 ± 328	2,400 – 3,430	8
Minerals			
Calcium (%)	1.19 ± 0.13	0.90 – 1.45	30
Phosphorus (%)	0.94 ± 0.06	0.81 – 1.10	30
Potassium (%)	0.883 ± 0.078	0.772 – 0.971	6
Chloride (%)	0.526 ± 0.092	0.380 – 0.635	8
Sodium (%)	0.313 ± 0.390	0.258 – 0.371	8
Magnesium (%)	0.168 ± 0.010	0.151 – 0.181	8
Sulfur (%)	0.280 ± 0.064	0.208 – 0.420	8
Iron (ppm)	360.54 ± 100	255.0 – 523.0	8
Manganese (ppm)	91.97 ± 6.01	81.70 – 99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10 – 64.50	8
Copper (ppm)	11.06 ± 2.50	8.09 – 15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52 – 4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04 – 2.09	8
Cobalt (ppm)	0.681 ± 0.14	0.490 – 0.780	4

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.52 ± 0.29	0.12 – 0.98	30
Cadmium (ppm)	0.10 ± 0.02	0.10 – 0.20	30
Lead (ppm)	0.32 ± 0.20	0.05 – 0.87	30
Mercury (ppm)	0.05 ± 0.01	0.05 – 0.06	30
Selenium (ppm)	0.37 ± 0.10	0.17 – 0.60	30
Aflatoxins (ppb)	<5.0		30
Nitrate nitrogen[b] (ppm)	20.80 ± 8.47	10.00 – 37.0	30
Nitrite nitrogen[b] (ppm)	0.28 ± 0.42	<0.10 – 2.10	30
BHA[c] (ppm)	3.77 ± 5.06	<2.00 – 22.0	30
BHT[c] (ppm)	1.17 ± 0.53	<0.10 – 3.00	30
Aerobic plate count (CFU/g)[d]	207,633 ± 280,205	22,000 – 1,200,000	30
Coliform (MPN/g)[e]	179 ± 233	<3.00 – 1,100	30
E. coli (MPN/g)	4.53 ± 7.28	<3.00 – 43.00	30
Total Nitrosoamines (ppb)[f]	8.62 ± 3.73	3.30 – 19.40	30
N-Nitrosodimethylamine (ppb)[f]	7.19 ± 3.02	3.00 – 14.00	30
N-Nitrosopyrrolidine (ppb)[f]	1.43 ± 1.43	0.30 – 5.40	30
Pesticides (ppm)			
α-BHC[g]	<0.01		30
β-BHC	<0.02		30
γ-BHC	<0.01		30
δ-BHC	<0.01		30
Heptachlor	<0.01		30
Aldrin	<0.01		30
Heptachlor epoxide	<0.01		30
DDE	<0.01		30
DDD	<0.01		30
DDT	<0.01		30
HCB	<0.01		30
Mirex	<0.01		30
Methoxychlor	<0.05		30
Dieldrin	<0.01		30
Endrin	<0.01		30
Telodrin	<0.01		30
Chlordane	<0.05		30
Toxaphene	<0.1		30
Estimated PCBs	<0.2		30
Ronnel	<0.01		30
Ethion	<0.02		30
Trithion	<0.05		30
Diazinon	<0.1		30
Methyl parathion	<0.02		30
Ethyl parathion	<0.02		30
Malathion[h]	0.16 ± 0.19	0.05 – 0.85	30
Endosulfan I	<0.01		30
Endosulfan II	<0.01		30
Endosulfan sulfate	<0.03		30

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU=colony forming unit
[e] MPN=most probable number
[f] All values were corrected for percent recovery
[g] BHC=hexachlorocyclohexane or benzene hexachloride
[h] Two lots contained more than 0.50 ppm

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Rats

Serum samples were taken from five male and five female rats at 6-month intervals during the 2-year study; however, to better evaluate the virological burden of the study, additional rats were introduced so that sera could be collected at additional time points. These samples were processed appropriately and submitted to Microbiological Associates, Inc. (Bethesda, MD) for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
ELISA	
PVM (pneumonia virus of mice)	6, 12, 14, 18, and 24 months
RCV (rat coronavirus)	6 months
RCV/SDA (rat coronavirus/sialodacryoadentitis virus)	8, 9, 12, 14, 18, and 24 months
Sendai	6, 8, 9, 12, 14, 18, and 24 months
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	6, 12, 18, and 24 months
KRV (Kilham rat virus)	6, 12, 18, and 24 months

Mice

Serum samples were taken from five male and five female sentinel mice at 6, 12, and 18 months during the study. Serum samples for the 24-month screening were obtained from five male and five female control mice. These samples were processed appropriately and submitted to Microbiological Associates, Inc. for determination of the virus antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	6 and 12 months
ELISA	
CARB (cilia-associated respiratory bacillus)	12 and 24 months
Ectromelia virus	6, 12, 18, and 24 months
GDVII (mouse encephalomyelitis virus)	6, 12, 18, and 24 months
LCM	24 months
MVM (minute virus of mice)	24 months
Mouse adenoma virus	6, 12, 18, and 24 months
MHV (mouse hepatitis virus)	6, 12, 18, and 24 months
Mycoplasma arthritidis	6, 12, and 24 months
Mycoplasma pulmonis	6, 12, and 24 months
PVM	6, 12, 18, and 24 months
Reovirus 3	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
Hemagglutination Inhibition	
K (papovavirus)	6, 12, 18, and 24 months
MVM	6, 12, and 18 months
Polyoma virus	6, 12, 18, and 24 months
Immunofluorescence Assay	
EDIM (Epizootic diarrhea of infant mice)	6, 12, 18, and 24 months
LCM	18 months
MVM	24 months

Results are presented in Table K1.

TABLE K1
Murine Virus Antibody Determinations for Rats and Mice in the 2-Year Feed Studies of C.I. Direct Blue 218

	Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Rats			
	6 months	10/10	Sendai
		9/10	RCV
	12 months	10/10	Sendai
		9/10	RCV/SDA
	18 months	10/10	Sendai
	24 months	0/8	RCV
Mice			
	6 months	0/10	None positive
	12 months	0/10	None positive
	18 months	0/9	None positive
	24 months	0/10	None positive

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-Ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-Methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4′-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

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TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
384	1,2,3-Trichloropropane

TR No.	CHEMICAL
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3′-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
398	Polybrominated Biphenyls
399	Titanocene Dichloride
400	2,3-Dibromo-1-propanol
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
404	5,5-Diphenylhydantoin
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4-Diamino-2,2-stilbenedisulfonic Acid
413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
416	*o*-Nitroanisole
417	*p*-Nitrophenol
418	*p*-Nitroaniline
419	HC Hellow 4
420	Triamterene
421	Talc
422	Coumarin
423	Dihydrocoumarin
424	*o*-Benzyl-*p*-chlorophenol
425	Promethazine Hydrochloride
427	Turmeric Oleoresin
428	Manganese (II) Sulfate Monohydrate
431	Benzyl Acetate
432	Barium Chloride Dihydrate
434	1,3-Butadiene
443	Oxazepam

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